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References: 1. Mod. Med. 23:107 (Nov. 15) 1955. 2. J.A.M.A. 154:213 (Jan. 16) 1954.

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STUDIES IN NEUROMUSCULAR FUNCTION

V. Effects of Anticholinesterase Compounds, d-Tubocurarine, and Decamethonium in Normal Subjects[1]

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The depression of neuromuscular transmission that occurs following the administration of neostigmine (1, 2) has been generally attributed to inhibition of muscle cholinesterase and accumulation at the motor end-plates of an excessive concentration of acetylcholine (ACh). In support of this idea is the prolongation by neostigmine of the end-plate potential recorded in experimental animals (3). However, it has been suggested (4) that neostigmine may have an additional direct action on neuromuscular transmission. Evidence for this is the production of an effect on muscle tension as rapidly as ACh following intra-arterial injection in the cat, a muscle twitch even when muscle cholinesterase has been inactivated by di-isopropyl fluorophosphate (5), and a contraction of denervated muscle (6).

Edrophonium was found to have more effect on neuromuscular transmission in experimental animals than would be expected from its weak anticholinesterase activity in vitro (7). Its action was also more rapid in onset and in time to peak effect than that of neostigmine (8). These observations suggested that edrophonium may produce its effects on neuromuscular transmission by a direct action rather than by inhibition of cholinesterase (4, 9). However, the finding that the action of both edrophonium and neostigmine was abolished in the cat by the prior administration of tetraethylpyrophosphate led others to maintain that their action is attributable to inactivation of cholinesterase (10).

There is evidence that d-tubocurarine produces neuromuscular block by inhibiting the access to the end-plates of ACh released from the motor nerve endings (11), without altering the resting membrane potential of the muscle fiber (12). In experimental animals it reduces not only the muscle response to nerve stimulation, but also the end-plate potential, indicating that it inhibits the action of ACh (13). d-Tubocurarine inhibits the neuromuscular action of injected ACh and neostigmine (14), the former in a competitive manner (15). This effect is more marked than the reduction in the muscle response to nerve stimulation, suggesting that injected ACh is blocked by d-tubocurarine more

[1] This work was supported by a contract between the U. S. Army Chemical Corps and the Johns Hopkins University, and was aided by grants from the Division of Neurologic Diseases and Blindness, National Institute of Health, U.S.P.H.S., and from the Smith, Kline, and French Foundation.

effectively than ACh released from motor nerve endings (16). The neuromuscular block produced by d-tubocurarine is reversible to a slight extent by ACh (17), and more completely by edrophonium (18) and neostigmine (19). The decurarizing action of edrophonium is greater than would be expected from its weak anticholinesterase activity in vitro (18). It has been proposed that the decurarizing activity, not only of edrophonium (18), but also of neostigmine (14), may be due mainly to a direct action, rather than to cholinesterase inhibition.

In most animal species, decamethonium produces neuromuscular block as a result of persistent depolarization of the motor end-plate region (20). It first stimulates the end-plate by depolarization. The depolarization of this region persists, rendering it unresponsive to ACh released from the nerve (21). This effect is not due to cholinesterase inactivation, although slight inhibition can be demonstrated in vitro (22). In some species, and in some muscles, decamethonium produces either a competitive or dual type of block, which may change with time. Thus, in the monkey, dog, or rabbit, there is transient increase in motor activity, suggestive of initial depolarization, followed by neuromuscular block which has the properties of the competitive type (23). In the cat, the block produced in the tibialis and gracilis, which are pale muscles, is of the depolarizing type, while in the soleus, a red muscle, it is initially depolarizing and then competitive (24). Most human muscles, including *m. abductor digiti quinti*, contain a mixture of pale and red fibers.

In the studies to be reported the effect in normal subjects of intra-arterially administered neostigmine, edrophonium, d-tubocurarine, and decamethonium on neuromuscular transmission and on the action of injected ACh was investigated. The methods employed were previously described (25). Neostigmine and edrophonium increased and prolonged the "prompt" depressant (depolarizing) action of ACh; d-tubocurarine inhibited this action, and decamethonium had no effect. The neuromuscular block produced by neostigmine and edrophonium appeared to be due mainly to anticholinesterase action, although edrophonium may have had an additional direct effect. The block produced by d-tubocurarine was of the competitive type, being reversible by neostigmine, tetanic nerve stimulation, and, to a lesser extent, ACh. Block of moderate degree due to decamethonium was of the non-competitive type, being intensified by neostigmine, while marked block had some properties of the competitive type, including reversal by small doses of neostigmine or tetanic nerve stimulation.

RESULTS

Anticholinesterase Agents

Effect on Neuromuscular Function

Neostigmine: The intra-arterial injection of neostigmine in doses of 0.3 to 1.5 mg. resulted, in each of 14 normal subjects, in progressive reduction in

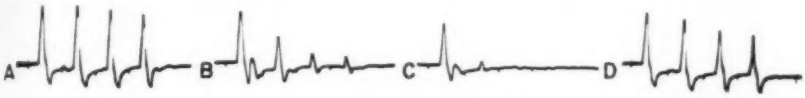

FIG. 1. Effect of edrophonium on muscle action potentials evoked by four maximal motor nerve stimuli at 40 msec. intervals. A, control. Amplitude of initial potential 6 mV. B, 2 seconds after 3 mg. edrophonium, which has produced progressive depression of all potentials, and repetitive firing after the first potential. C. 4 seconds after the injection. D, 8 minutes after the injection. (Normal subject P. D.).

amplitude and duration of the muscle action potential responses to successive nerve stimuli. The response to the first stimulus of a train was moderately reduced in five subjects, slightly reduced in four and unchanged in five. The responses to the second and subsequent stimuli were reduced in all, usually in a progressive manner, although occasionally that to the second stimulus to a greater extent than to the third and fourth. The average reduction in amplitude of the first response following 1 mg. of neostigmine was 20 per cent, and of the fourth 70 per cent. The injected arm became weaker in all subjects. Usually repetitive firing of the muscle fibers in response to the first stimulus could be demonstrated, as well as numerous local fasciculations. These effects of neostigmine have been previously described (2). The smallest dose which produced this effect was 0.02 to 0.05 mg.

The mean latent period between neostigmine administration and onset of detectable change in the muscle action potentials was 12 (S.E. 2.4) seconds, and the interval between administration and maximal effect was 44 (S.E. 7) seconds. The duration of effect varied with the dose, being from one minute after 0.02 mg. to more than two hours after 1.5 mg.

Edrophonium: The intra-arterial injection of 0.2 to 15 mg. of edrophonium resulted in reduction in amplitude of the muscle action potentials in each of 14 normal subjects (Fig. 1). The pattern of depression resembled that following neostigmine, but the effect on the initial potential was greater. Following large doses of edrophonium the muscle action potentials were sometimes completely abolished. When this occurred progressive depression of the responses was observed during the period of recovery. The effect of edrophonium developed more rapidly than that of neostigmine, beginning within a mean of 9 (S.E. 1.1) seconds after injection. The maximum effect was reached sooner, occurring 24 (S.E. 3.7) seconds after injection. Following moderate depression by edrophonium the potentials returned to their original amplitude in from one to 20 minutes; i.e., in one tenth the time required for recovery from comparable depression by neostigmine.

Effect on the "prompt" depressant Action of ACh

Neostigmine: The prior administration of neostigmine resulted in a marked increase in the degree and duration of the "prompt" depression produced by

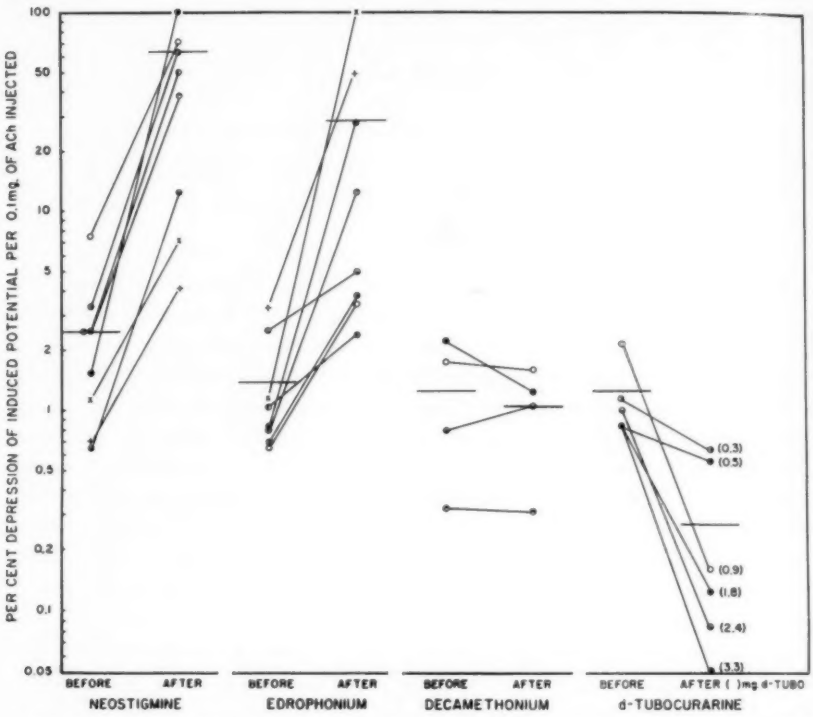

FIG. 2. Increase in the "prompt" depressant action of ACh on the muscle action potential response to a single maximal nerve stimulus by neostigmine (0.3 to 1.2 mg.) and edrophonium (3 to 12 mg.); lack of effect of decamethonium; and inhibition by d-tubocurarine. The doses of decamethonium administered were 0.1 to 0.5 mg., which produced 58 to 70 per cent reduction in amplitude of the action potential, while d-tubocurarine produced 6 to 54 per cent reduction. The horizontal lines represent averages.

ACh. The depression of the first of a series of evoked potentials increased from a mean of 2.5 per cent to 62 per cent per 0.1 mg. of ACh (Fig. 2). In contrast to the even depression of successive action potentials produced by ACh prior to neostigmine, ACh administered after neostigmine resulted in progressive depression (Figs. 3 and 4). This was noted not only when there was some decline in successive action potentials as a result of neostigmine administration, but also when the action potentials had either been unaffected by the dose of neostigmine administered, or had returned to their original amplitude. The depression of the fourth of a series of evoked potentials was close to 100 per cent per 0.1 mg. of ACh. The time between ACh administration and maximal depression was not significantly altered, but the duration of the effect was in-

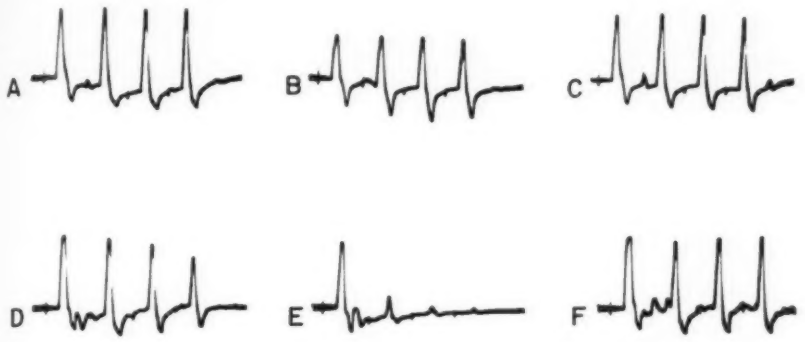

FIG. 3. Increase in the "prompt" depressant effect of ACh by neostigmine. A, control muscle action potentials in response to four nerve stimuli delivered at 40 msec. intervals. Amplitude of initial potential 5.5 mV. B, maximum depression 10 seconds after the intra-arterial injection of 1 mg. ACh. C, recovery 48 seconds after injection. D, control response 30 minutes after the intra-arterial injection of 1.5 mg. neostigmine. E, maximum depression 10 seconds after the injection of 0.025 mg. ACh. F, recovery 60 seconds after injection. (Normal subject P. D.).

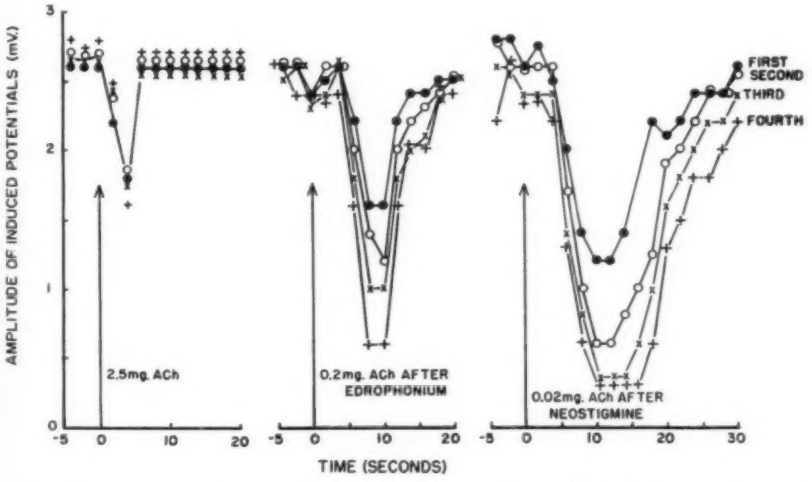

FIG. 4. Potentiation and prolongation by intra-arterially injected edrophonium or neostigmine of the "prompt" depressant action of ACh on muscle action potentials evoked by a train of four nerve stimuli (40 msec. apart) repeated every two seconds. Twelve minutes after the administration of edrophonium (12 mg.), and, on another occasion, 30 minutes after neostigmine (1.5 mg.), ACh was injected in doses which had previously had no effect on evoked potentials. (Normal subject J. P.).

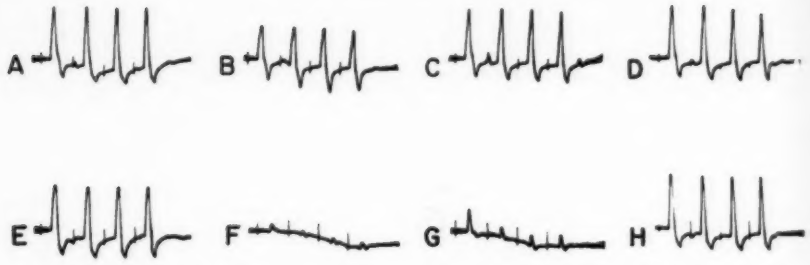

FIG. 5. Increase in the "prompt" depressant effect of ACh by edrophonium. A, control muscle action potentials in response to four nerve stimuli delivered at 40 msec. intervals. B, maximum depression 10 seconds after the intra-arterial injection of 1 mg. ACh. C, recovery 12 seconds after injection. D, 48 seconds after injection. E, control response 12 minutes after the intra-arterial injection of 3 mg. edrophonium. F, maximum depression 5 seconds after 0.5 mg. ACh. G, 12 seconds after injection. H, 48 seconds after the injection. (Normal subject P. D.).

creased several fold. The increased reactivity to ACh was maximal 3 to 14 minutes after the injection of neostigmine, and diminished gradually over a period of one to three hours. The repetitive response to a single nerve stimulus and the muscular fasciculations produced by neostigmine were diminished or abolished by ACh.

Edrophonium: The administration of edrophonium resulted in a moderate increase in the "prompt" depressant effect of ACh. The effect on the first of a series of potentials increased from a mean of 1.4 to 29 per cent depression per 0.1 mg. of injected ACh (Fig. 2). As with neostigmine, the prior administration of edrophonium resulted in progressive depression of the successive muscle potentials by ACh (Figs. 4 and 5). The mean depression of the fourth potential of a train was 41 per cent per 0.1 mg. of ACh. The duration of the "prompt" depressant effect of ACh was increased, but to a lesser extent than following neostigmine. Edrophonium continued to enhance the action of injected ACh even after the effect of the edrophonium on the electromyogram had worn off. The duration of its influence on the action of ACh was approximately 20 minutes; i.e. one fifth that of neostigmine.

Effect of Edrophonium on the Action of Neostigmine

When 0.3 to 1.5 mg. of neostigmine was injected 15 to 30 minutes after edrophonium, its effect on the evoked potentials was partially or completely blocked. The effect of neostigmine on reactivity to injected ACh was reduced, but never completely prevented.

d-Tubocurarine

Effect of neuromuscular function: *Dose-effect relationship:* The effect of the intra-arterial injection of d-tubocurarine on muscle action potentials evoked by

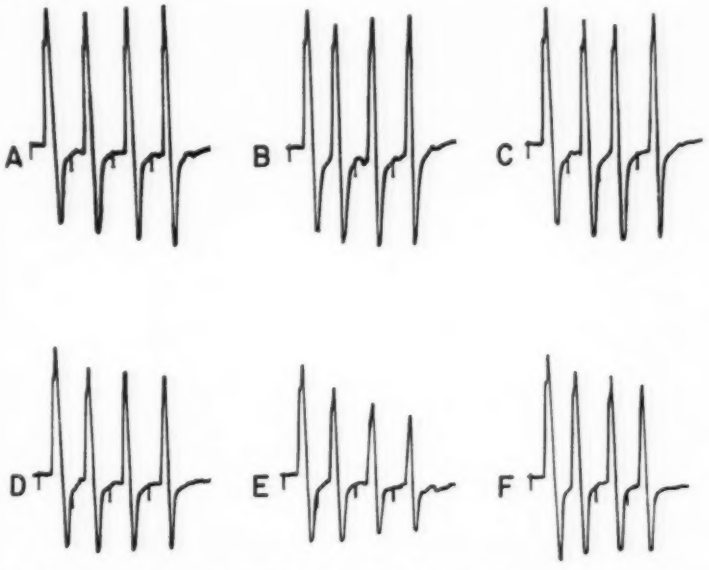

FIG. 6. Depressant effect of successive doses of d-tubocurarine on neuromuscular transmission. A, control muscle action potentials in response to four nerve stimuli at 40 msec. intervals. B, effect of the intra-arterial injection of 0.45 mg. d-tubocurarine. C, effect of an additional 0.6 mg. D, effect of an additional 0.9 mg. E, effect of an additional 1.5 mg. F, decrease in the effect 4 minutes later. (Normal subject J. R.).

nerve stimulation was studied in 13 subjects. The administration of 0.15 mg. usually had no effect on the initial potential of a train, but produced slight progressive depression of subsequent potentials. With larger doses there was variable depression of the initial potential; 0.3 mg. produced between 0 and 60 per cent reduction, 0.6 mg. 30 to 60 per cent reduction, and 0.9 to 1.5 mg. 30 to 70 per cent reduction. As the dose was increased there was also increasing depression of successive potentials of a train (decrement), the fourth potential being reduced 10 to 40 per cent more than the first (Fig. 6). The action of d-tubocurarine began a mean of 13 (S.E. 1.8) seconds after injection and was maximal in a mean of 45 (S.E. 4.9) seconds. The latter potentials sometimes continued to decline slightly during the next one or two minutes. With repeated injections there was usually increasing depression and decrement, but successive injections had a diminishing effect. In one subject repeated doses of 1.5 mg. produced little further depression after the initial injection.

Effect on the response to paired nerve stimuli: Prior to d-tubocurarine the responses to a single conditioning stimulus followed by a test stimulus delivered at intervals greater than 16 msec. were equal (26). Following the administration of sufficient drug to reduce the amplitude of the response to

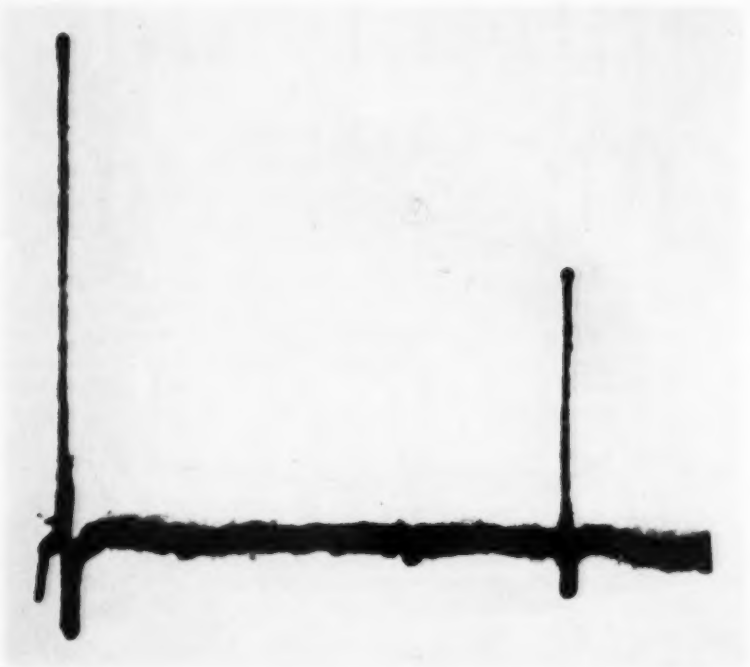

FIG. 7. Depressant effect of d-tubocurarine on the muscle action potentials in response to two stimuli delivered at a one second interval. Amplitude of response before d-tubocurarine was 9.6 mV. Following the intra-arterial injection of 0.9 mg. d-tubocurarine the first response here is 1.9 mV, the second 1.0 mV. (Normal subject J. N.).

the conditioning stimulus by more than 20 per cent, the response to a test stimulus delivered within 10 seconds was reduced to a greater degree (Fig. 7); i.e. the response to the first impulse was followed by a period of increased neuromuscular block. The increase in the block was maximal one half to one second after the initial response, and did not disappear until approximately 10 seconds later (Fig. 8).

Effect on the response to repetitive nerve stimulation: Prior to d-tubocurarine administration the action potential responses to repetitive nerve stimulation delivered at frequencies of from 5 to 25 per second for periods up to one minute were equal (26). Following d-tubocurarine, there was a decline in the response to repetitive stimulation, with a characteristic pattern consisting of rapid fall in the amplitude of the responses, followed by rapid, partial recovery and then by slow progressive decline (Fig. 9). The degree and rate of

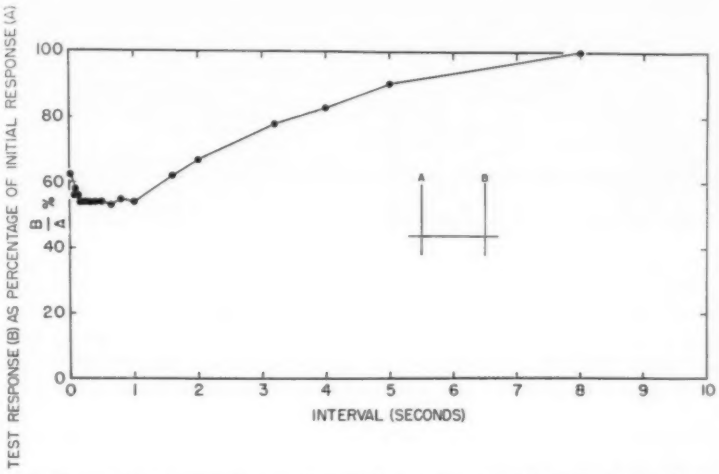

FIG. 8. Effect of d-tubocurarine on the muscle action potential response to a single (test) stimulus delivered at varying intervals following a single (conditioning) stimulus. The ordinate is the percentage of the first response (A) represented by the second response (B). Prior to d-tubocurarine the amplitude of the potentials in response to the two stimuli was equal, and measured 9.6 mV. Following the intra-arterial injection of 0.9 mg. d-tubocurarine, the test stimulus, which produced a muscle potential response of 1.9 mV, was followed by depression of the response to the conditioning stimulus. (Normal subject J. N.).

FIG. 9. The effect of d-tubocurarine on the muscle action potentials in response to repetitive nerve stimulation at a frequency of 25 per second. Prior to d-tubocurarine the amplitude of the initial potential was 9.6 mV, and, after 1.2 mg. intra-arterially, it is 3.8 mV. (Normal subject J. N.).

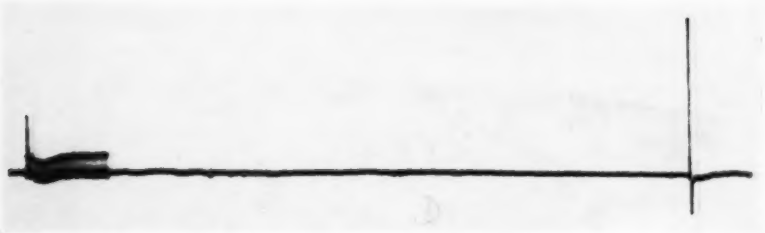

FIG. 10. Partial reversal by repetitive nerve stimulation of neuromuscular block due to d-tubocurarine (post-tetanic decurarization). The conditioning stimulus is a train of 121 impulses at 200 per second, and the single test stimulus is delivered 5 seconds later. The amplitude of the muscle action potential response before d-tubocurarine was 9.6 mV. Following the intra-arterial injection of a total of 2.4 mg. d-tubocurarine, the amplitude of the first of the train is 1.1 mV, and of the test response 3.7 mV. (Normal subject J. N.).

depression was greater following larger doses of d-tubocurarine and following more rapid rates of stimulation.

Effect on the response to a single stimulus or a tetanus delivered after a tetanus: Prior to drug administration repetitive nerve stimulation at any frequency was not followed by an increase in the action potential response to a single stimulus (26). Following partial neuromuscular block by d-tubocurarine, repetitive nerve stimulation was followed by a period during which there was a decrease in the block, as indicated by an increase in the amplitude of the response to a single stimulus (Fig. 10). This post-tetanic decurarization began half a second after the tetanus, reached a maximum in one to four seconds, and lasted more than ten seconds (Fig. 11). Its degree and duration increased with the frequency and duration of the tetanus, within the limits studied (Fig. 11), even though the muscle action potentials decreased rapidly during tetani delivered at high frequencies, and disappeared during a tetanus at 200 per second. When the conditioning tetanus was followed by another tetanus, instead of a single stimulus, the first response to the second train of stimuli was potentiated in the same manner as the single response, but subsequent responses declined more rapidly than did those to the conditioning train.

Effect on the action of ACh: The administration of d-tubocurarine greatly inhibited and delayed the onset of both the stimulating (twitch producing) and "prompt" depressant effects of ACh in each of four subjects studied (Figs. 2 and 12). The degree of inhibition of ACh action was directly proportional to the dose of d-tubocurarine and varied only roughly with the degree of depression of the action potentials produced by this drug. Each mg. of injected d-tubocurarine inhibited the action of approximately 25 mg. of injected ACh. The latent period between the injection of ACh and the onset of its depressant action was prolonged by 5 to 10 seconds following d-tubocurarine, and the duration

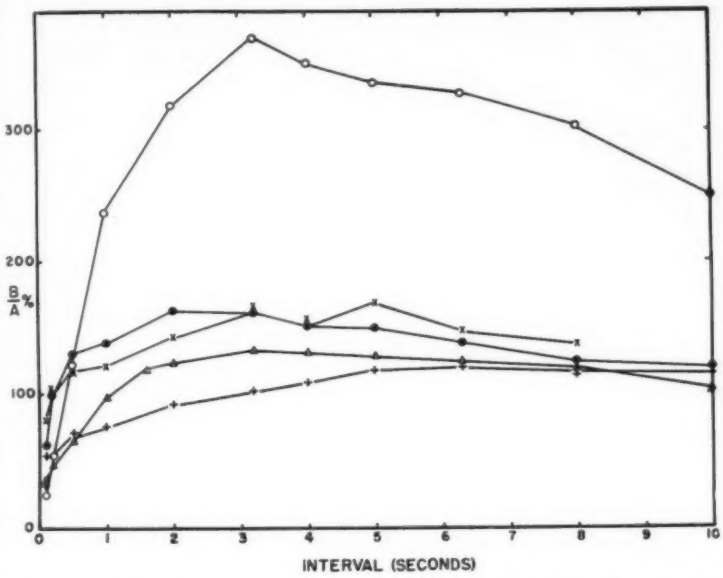

FIG. 11. Effect in a partially curarized normal subject (J. N.) of varying the duration and frequency of the tetanus on the degree of post-tetanic decurarization. The latter is expressed as the percentage of the response to the initial stimulus of the tetanus (A) represented by the response to the single stimulus (B) delivered at varying intervals after the tetanus. The conditioning tetani employed were:

○——○ 121 impulses at 200 per second,
●- - -● 61 impulses at 200 per second,
×- - -× 31 impulses at 200 per second,
+- - -+ 13 impulses at 200 per second,
△- - -△ 56 impulses at 25 per second.

of the depressant action was usually reduced slightly. The inhibition of the depressant action of ACh was usually greater on the initial potential of a train than on the latter potentials (Fig. 12).

Effect of ACh on d-tubocurarine block: ACh, in doses of 5 to 20 mg., produced either very slight and transient, or no decurarization. When present, it occurred immediately following the period of "prompt" depression and lasted up to 15 to 20 seconds (Fig. 12). The rate of spontaneous recovery from the effect of d-tubocurarine was either unaffected or was slightly accelerated for a period of 10 seconds. When decurarization did occur it was usually greater in the latter potentials of a train, resulting in a decrease in the decrement that was present. The maximum degree of decurarization observed was 7 per cent of the initial amplitude of the first potential of a train and 14 per cent of the fourth potential.

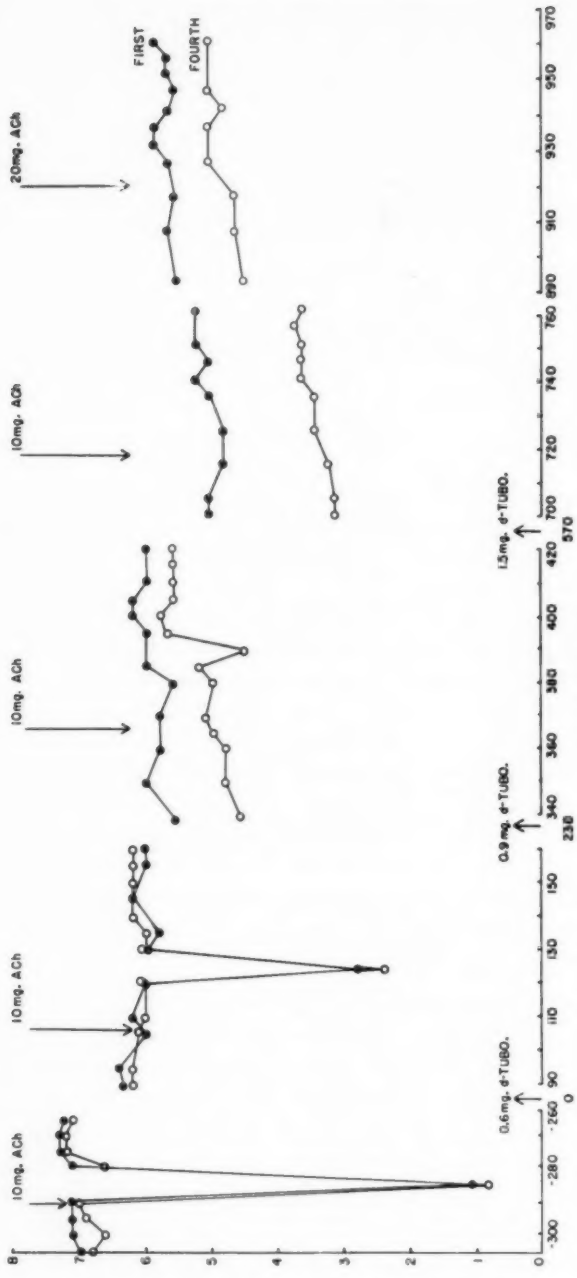

FIG. 12. Inhibition and delay by d-tubocurarine of the "prompt" depressant action of ACh on muscle action potentials in response to a train of four stimuli (40 msec. apart) evoked every 2 seconds. The ACh produced only slight decurarization. (Normal subject J. R.).

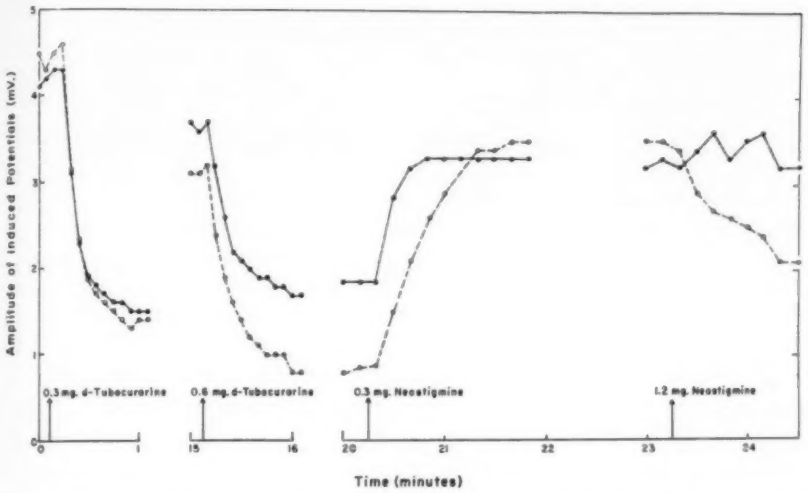

FIG. 13. Reversal by neostigmine of the depressant effect of d-tubocurarine on evoked muscle action potentials. The amplitude of the first (●——●) and fourth (○- - -○) potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted. A larger dose of neostigmine then produced depression of the latter potentials of the train. (Normal subject C. H.).

Reversal of d-tubocurarine block by neostigmine: The decurarizing action of neostigmine was much greater and more prolonged than that of ACh. The administration of 0.3 mg. of neostigmine following depression of evoked potentials by d-tubocurarine resulted in progressive increase in their amplitude, beginning in approximately 15 seconds and reaching a maximum 100 seconds after injection (Fig. 13). The depression due to d-tubocurarine was fully corrected in most instances. The subsequent injection of 1.2 mg. of neostigmine produced depression of the latter potentials of a train, and repetitive firing after the initial potential, similar to the effect of neostigmine administered alone, though not as marked.

Decamethonium

The type of neuromuscular block produced by decamethonium varied with the degree of block. When moderate, it had the properties of the non-competitive type (25, pp. 118–19); when marked, it had many of the properties of the competitive type (page 118).

Effect on neuromuscular function: *Dose-effect relationship:* Graded doses of decamethonium were administered intra-arterially to ten normal subjects. The injection of 0.05 mg. resulted in a burst of motor activity, consisting of potentials 50 to 100 microvolts in amplitude which could be recorded by concentric needle electrodes and which were usually accompanied by a few transient

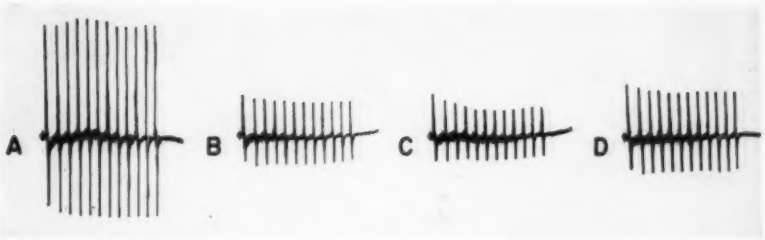

FIG. 14. Depressant effect of decamethonium on evoked muscle action potentials. A, control response to 12 nerve stimuli 40 msec. apart. Amplitude of initial potential 7.8 mV. B, 25 seconds after the intra-arterial injection of 0.05 mg. decamethonium. C, 95 seconds after injection, showing increase in decrement of the successive potentials. D, 285 seconds after injection. (Normal subject C. W.).

fasciculations. This activity lasted approximately 5 seconds. It was immediately followed by a 40 to 85 per cent reduction in the amplitude of muscle action potentials evoked by nerve stimulation. The depressant effect began an average of 12 (S.E. 1.6) seconds after injection, and reached its maximum in 32 (S.E. 4.4) seconds. The degree of reduction of successive potentials of a train was either equal, or was five to ten per cent greater in the fourth than in the first potential (Fig. 14). The development of progressive depression of the successive potentials was greater after larger doses of drug, or after repeated injections of smaller doses. Following 0.5 mg. of decamethonium, which resulted in a 95 per cent reduction in the initial potential, there was fairly marked progressive decrease in amplitude of the latter potentials evoked by a long train (41 nerve stimuli at 25 per second).

Effect on the response to paired nerve stimuli: Following the administration of 0.05 mg. decamethonium the responses to a conditioning stimulus and a test stimulus delivered to the nerve at an interval of more than 16 msec. were evenly depressed. Following larger doses of decamethonium (e.g. 0.2 mg.) there was greater reduction in the muscle potential response to the second of the two stimuli delivered at an interval between 16 msec. and 10 seconds.

Effect on the response to a single stimulus delivered after a tetanus: Following moderate neuromuscular block produced by decamethonium there was no decrease in the degree of block following tetanic nerve stimulation. However, after marked neuromuscular block by larger doses of decamethonium, there was a moderate increase in the response to a single stimulus delivered to the nerve 1 to 10 seconds after the tetanus (Fig. 15). This post-tetanic facilitation was much less marked than that observed during partial curarization (Fig. 16). Following the administration of sufficient decamethonium to reduce the action potentials to 8 per cent of their normal amplitude, tetanic nerve stimulation resulted in an increase in the response to a single stimulus by 78 per cent. The time course of the post-tetanic facilitation was similar to that observed following d-tubocurarine.

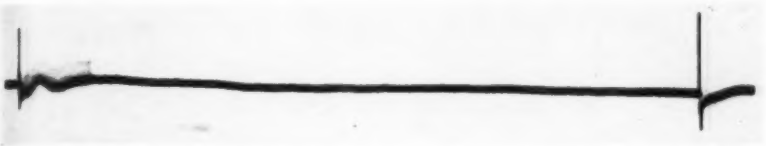

FIG. 15. Partial decrease in neuromuscular block due to decamethonium following a tetanus applied to the nerve (post-tetanic facilitation). The conditioning stimulus is a train of 121 impulses at 200 per second, and the single test stimulus is delivered 5 seconds later. The amplitude of the first potential of the train is 0.5 mV, and of the test response 0.75 mV. Prior to decamethonium (0.5 mg. intra-arterially) the amplitude of these potentials was 5.6 mV. (Normal subject P. M.).

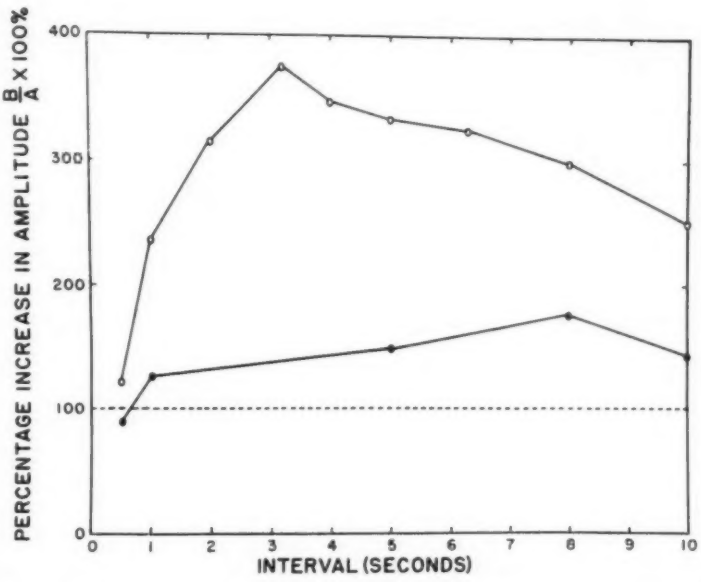

FIG. 16. Comparison of the decrease following a tetanus applied to the nerve in neuromuscular block due to d-tubocurarine (○——○) and decamethonium (●——●) in a normal subject (J. N.). The degree of post-tetanic facilitation of the muscle action potential response (abscissa) is expressed as the per cent increase in amplitude of the response (B) to a single stimulus delivered at varying intervals after the tetanus (amplitude of initial potential of tetanus = A).

Lack of effect on the action of ACh, and failure of ACh to reverse decamethonium block: The administration of decamethonium in doses up to 0.25 mg., which produced a 75 per cent reduction in amplitude of evoked muscle action potentials, had no effect on the stimulating or "prompt" depressant effect of ACh on these potentials. The "prompt" depressant effect of ACh was unchanged as measured by per cent reduction in the action potentials (Figs. 3

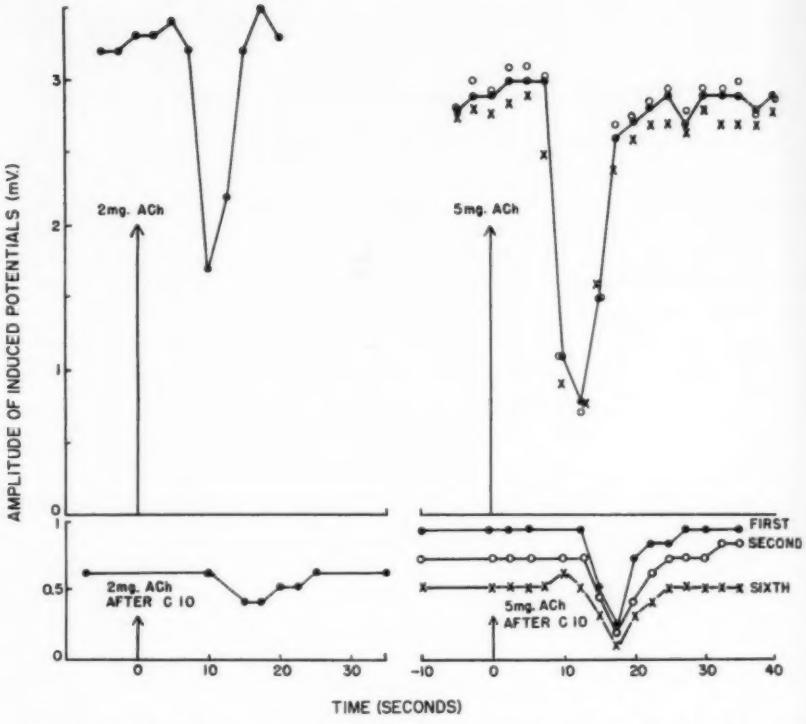

FIG. 17. Lack of effect of intra-arterially injected decamethonium (0.1 mg.) on the prompt depressant action of ACh on muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds. The action of ACh was slightly delayed, but the per cent reduction of potentials by ACh was unchanged. (Normal subject E. M.).

and 17). The reduction in mV of these potentials (already depressed by decamethonium) was less. Successive potentials of a train were equally reduced by ACh, so that any progressive depression produced by decamethonium was preserved (Fig. 17). The onset of "prompt" depression by ACh, and the time to maximum depression, were delayed by five to ten seconds following decamethonium, but the duration of depression was usually unchanged. In no instance did ACh, administered in doses from 0.6 to 20 mg., reverse the decamethonium block or accelerate recovery from this block.

Effect of neostigmine on decamethonium block: The effect of neostigmine administration on the amplitude of evoked potentials following their depression by decamethonium varied with the dose of neostigmine and the dose or degree of depressant effect of decamethonium. Following marked depression by 0.1 mg. of decamethonium the administration of a small dose of neostigmine (0.1 mg.) reversed the decamethonium depression (Fig. 18). Larger doses of

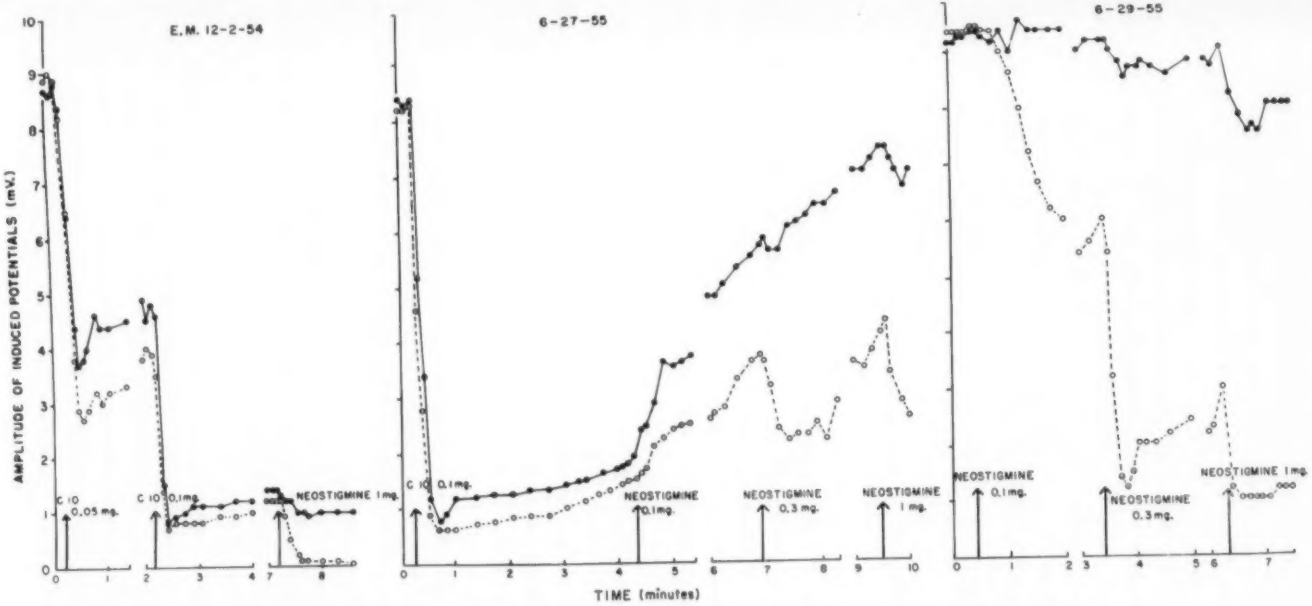

FIG. 18. Effect of neostigmine on the depression of evoked muscle action potentials produced by decamethonium.

Left: Intensification of decamethonium block by 1 mg. neostigmine.

Middle: Reversal of decamethonium block by smaller doses of neostigmine, and inhibition of the depressant effect of these doses on evoked potentials.

Right: Depressant effect of neostigmine alone.

The amplitude of the muscle action potentials in response to the first (●——●) and fourth (○- - -○) of a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted before and after the intra-arterial injection of each drug. (Normal subject E. M.)

neostigmine (e.g. 0.3 or 1 mg.) produced further depression, but this was less marked than with neostigmine alone (Fig. 18). Following moderate depression by 0.05 mg., or less, of decamethonium the administration of neostigmine in doses of 0.1 mg. or more, produced further depression, especially of the latter potentials of a train, but this was less marked than with neostigmine alone.

Effect of edrophonium on decamethonium block: Edrophonium, in doses of 0.1 to 10 mg. did not reverse decamethonium block of moderate degree or accelerate recovery from this block. The depressant effect of edrophonium was moderately reduced by the prior administration of decamethonium.

Effect of prior administration of ACh and choline on the depressant action of decamethonium: The prior administration of sufficient ACh to produce "late" depression of induced potentials had no effect on the depressant action of decamethonium, but the prior administration of large doses of choline resulted in a slight reduction in this action.

DISCUSSION

In man the effects of *neostigmine* on neuromuscular transmission and on the action of ACh seem to be due mainly to the inhibition of cholinesterase. The depressant effect of neostigmine on the muscle potentials evoked by nerve stimulation was slower in onset than that of ACh, beginning 12 seconds after injection and becoming maximal in 44 seconds, compared to 5 and 7 seconds for ACh (27). In contrast to the even depression of the successive responses to repetitive nerve stimulation produced by ACh, neostigmine produced little change in the initial potential of a train, and progressive depression of subsequent responses. This effect can be accounted for by the inhibition of muscle cholinesterase, resulting in accumulation of endogenous ACh at the neuromuscular junction following the arrival of successive impulses at the motor nerve endings. In some instances the second potential of a train was depressed to a greater extent than the third and fourth potentials. This may be due to the opposing influences of accumulation of ACh following the arrival of each stimulus at the motor nerve ending, and its removal by diffusion and hydrolysis by uninhibited cholinesterase.

When ACh was administered after neostigmine it produced progressive, rather than even, "prompt" depression of the successive potentials in response to repetitive nerve stimulation. This may be explained by the addition of the ACh released by each nerve impulse to that which was injected. The similarity between the progressive depression of successive potentials in response to repetitive nerve stimulation produced by neostigmine and by ACh administered after neostigmine supports existing evidence that ACh is the mediator of neuromuscular transmission, and that its accumulation due to inhibition of cholinesterase is responsible for the major effect of neostigmine on neuromuscular transmission.

The effect of *edrophonium* resembled that of neostigmine, except that its action was more rapid in onset and shorter in duration. In addition it caused a greater reduction in the initial potential of a train than did neostigmine. The effect of edrophonium on the "prompt" depressant action of ACh was similar to that of neostigmine. When edrophonium was administered prior to neostigmine, the neuromuscular effects of the latter drug were partially or completely blocked, probably as a result of combination of edrophonium with cholinesterase. These observations indicate that the action of edrophonium is also due principally to cholinesterase inactivation, but the time course and effect on the initial potential suggest that there may be, in addition, a direct effect on neuromuscular transmission.

d-Tubocurarine readily blocked the neuromuscular effects of injected ACh following doses which produced little or no depression of the muscle potentials. Thus, as in experimental animals (16), d-tubocurarine blocked the action of injected ACh more readily than the response to nerve stimulation. As in experimental animals, the slight decurarizing action of ACh was in contrast to the marked decurarizing action of neostigmine. If the latter is mediated by cholinesterase inactivation, the difference might be due to more ready access to the end-plate of ACh released from motor nerve endings than of injected ACh. The time course of the decurarizing action of neostigmine, and the more marked effect on the latter potentials of a train than on the initial potential, are compatible with cholinesterase inactivation. However, a direct mode of action cannot be excluded.

During d-tubocurarine block the "prompt" depressant and decurarizing actions of ACh were greater on the latter potentials of a train than on the initial potential. This is in contrast to the even depression produced by ACh in normal subjects, and is surprising in view of the evidence that the per impulse output of ACh from the nerve ending decreases during repetitive stimulation (28, 29). It may be due to slight depression of muscle cholinesterase by d-tubocurarine (30) or ACh, resulting in the accumulation of ACh released during repetitive nerve stimulation. Some progressive decline of induced potentials was noted following the administration of ACh alone during very rapid nerve stimulation (27). Following d-tubocurarine block, the response of the motor end-plates may more readily reflect small variations in ACh concentration.

Evidence obtained in experimental animals indicates that during repetitive nerve stimulation there is a progressive decrease in the amount of ACh released from the motor nerve endings, as reflected by a progressive decrease in size of the end-plate potentials (28, 29). Despite this decrease, the amount of transmitter released normally remains sufficient to produce a maximal response of the muscle. However, when the threshold of the end-plate to the transmitter effect is raised by a competitive blocking agent such as d-tubocurarine, this amount of transmitter is no longer sufficient to depolarize all the end-plates

and there is a decrease in the muscle response. Numerous observations in experimental animals have indicated that while d-tubocurarine competitively blocks the action of ACh on the end-plates, it does not interfere with the release of ACh from the motor nerve endings (31). Therefore, the observation in partially curarized man of depression of the response to the second of paired stimuli, progressive decline in the response to repetitive nerve stimulation, post-tetanic facilitation and subsequent more rapid decline in the response to tetanic stimulation, is believed to reflect variations in ACh output from motor nerve endings following nerve stimulation. The variations are unmasked by the competitive (ACh inhibitory) nature of the curare block. The more rapid decline in the response to repetitive nerve stimulation following post-tetanic facilitation may be due to depletion during the brief period of facilitation of ACh stored in the motor nerve endings. The time course of the changes in muscle action potentials evoked by nerve stimulation is very similar to that of the end-plate potentials recorded in curarized experimental animals (28, 29).

The time course of the depression of the response to the second of paired stimuli in the curarized subject is different from that produced by neostigmine (2). Following the latter drug, the depression, which is attributable to cholinesterase inhibition and accumulation of ACh, is maximal when the stimuli are less than 16 msec. apart, and disappears when the stimuli are more than 100 msec. apart.

The properties of d-tubocurarine block in normal subjects resemble in many ways the neuromuscular block present in patients with myasthenia gravis, but there are also a number of differences. The similarities include depression of the response to the second of paired stimuli, the pattern of response to repetitive nerve stimulation, and post-tetanic facilitation. These similarities suggest that the defect in myasthenia gravis may be a competitive (ACh inhibitory) block. Evidence has been provided that the defect in myasthenia gravis may be due to the production of a competitive block by choline, or by a derivative of choline, formed following the hydrolysis of ACh during neuromuscular transmission (32). The reversal of d-tubocurarine block by neostigmine and edrophonium resembles the reversal of the myasthenic block by these compounds. The feeble decurarizing action of ACh, however, is in contrast to the more striking repair of the myasthenic block by this compound. Whereas in the curarized normal subject the decurarizing action of ACh was much less than that of neostigmine, in the myasthenic patient the maximal repair attained following ACh was either the same as, or only slightly less than that produced by neostigmine. This suggests that the competitive block present in the myasthenic patient, and that produced by d-tubocurarine in the normal subject, may be exerted at different sites on the end-plate, and that the former may be more easily displaced from the end-plate by ACh than is d-tubocurarine. In both the curarized normal subject and the myasthenic patient the reparative action

of ACh was greater on the latter potentials of a train than on the initial potential, although the difference was much more striking in the myasthenic patient.

In the myasthenic patient there can be appreciable reduction in the amplitude of the response to successive nerve stimuli when the first response is not blocked, or is only slightly blocked (32), while in the curarized normal subject this does not occur unless there is moderate block of the first response. Thus, at comparable degrees of depression of the initial potential of a train, the degree of progressive depression of the second and subsequent potentials was greater in the myasthenic patient than in the curarized normal subject.

In both d-tubocurarine block and myasthenia gravis the "prompt" depressant action of ACh on evoked potentials was reduced. In the curarized normal subject this action of ACh on the initial potential of a train was inhibited to a slightly greater extent than on subsequent potentials. In the myasthenic patient, the action of ACh on the latter potentials of a train was inhibited to a much greater extent than on the initial potential. The stimulating (twitch-producing) effect of injected ACh was also blocked in the curarized normal subject to a marked degree, whereas in the myasthenic patient it was only slightly less than in normal subjects.

These observations provide additional evidence that the competitive block observed in patients with myasthenia gravis is increased to a greater degree during and after neuromuscular transmission than is that due to partial curarization. This could be explained by the formation of choline following hydrolysis of ACh, and the production of a competitive block by this compound or one of its derivatives. In the patient with mild myasthenia, no block may be clearly evident until voluntary or electrical stimulation of the nerve has occurred. In the curarized normal subject, on the other hand, the competitive block is influenced little, if at all, by neuromuscular transmission. The progressive decline in response to nerve stimulation observed in the curarized normal subject is believed to reflect normally occurring variations in ACh output during repetitive nerve stimulation. Such variations very likely occur in the myasthenic patient too, but their effect is magnified by the increasing ACh-inhibitory block which develops in this disease following nerve stimulation.

The properties of the neuromuscular block produced by *decamethonium* varied with the degree of block. Following moderate block, there was even depression of the response to paired or repetitive nerve stimuli, and no post-tetanic facilitation, indicating that the end-plates were not reflecting the variations in ACh output from the motor nerve endings that normally occur following nerve stimulation. In addition, the block was intensified by neostigmine, even in small doses. The failure of decamethonium to potentiate the "prompt" depressant action of ACh or neostigmine indicates that decamethonium has negligible anticholinesterase action. It also suggests that the site of depolariza-

tion of the end-plate by decamethonium is distal to the site of action of ACh. This is compatible with findings in the experimental animal that decamethonium is capable of depolarizing the muscle membrane near the end-plate (20).

Following marked decamethonium block there was progressive depression of the response to paired or repetitive nerve stimuli, post-tetanic facilitation of mild degree, and partial reversal of the block by small doses of neostigmine, indicating a competitive type of block at some of the motor end-plates. However, in contrast to the effect of d-tubocurarine, the "prompt" depressant (depolarizing) action of ACh was only slightly inhibited. Since decamethonium produces an initial burst of motor activity, followed by depression of successive evoked potentials which becomes progressive over a period of one to two minutes, it appears to have a dual mode of action, initially depolarizing and then, in part, competitive. The effects of decamethonium in man resemble in many ways the effects of choline (27).

SUMMARY

1. In normal subjects the intra-arterial administration of neostigmine and edrophonium produced progressive depression of the successive muscle action potentials evoked by repetitive nerve stimulation, and increase and prolongation of the "prompt" depressant effect of ACh. These effects are compatible with action mainly as anticholinesterase agents. Edrophonium had more rapid onset of action than neostigmine and more depressant effect on the initial potential of a train, indicating that it may have an additional direct effect on transmission.

2. d-Tubocurarine produced progressive depression of successive evoked potentials which was reversible by neostigmine, tetanic nerve stimulation, and, to a lesser extent, ACh. It markedly inhibited the "prompt" depressant effect of ACh. These actions are those of a competitive (ACh inhibitory) block. This block resembled in many ways the defect present in myasthenia gravis, but differed in that the neuromuscular block in this disease increases to a greater extent following nerve stimulation.

3. Decamethonium produced a transient burst of motor activity followed by depression of evoked potentials. When the block was moderate in degree, it had the properties of the non-competitive type (even reduction of successive potentials of a train, and enhancement of the block by neostigmine). When the block was marked, it had many of the properties of the competitive type (progressive reduction of successive potentials of a train, and reversibility by neostigmine).

4. The relationship of these results to knowledge of the pathogenesis of myasthenia gravis is discussed.

ACKNOWLEDGMENTS

The authors are indebted to Leroy H. Warthen and Olive L. Smith for their valuable technical assistance.

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STUDIES IN NEUROMUSCULAR FUNCTION

VI. Effects of Anticholinesterase Compounds, d-Tubocurarine, and Decamethonium in Patients with Myasthenia Gravis[1]

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The administration of neostigmine to patients with myasthenia gravis results in improvement in the weakness, abnormal fatigability, and impairment of neuromuscular transmission (1) that characterize this disease. These effects are generally ascribed to the anticholinesterase action of neostigmine, but a direct "decurarizing" action may be involved (2). Edrophonium produces a more transient improvement in strength (3), which reportedly may include muscles that fail to respond to neostigmine (4). Since this improvement is greater than one might anticipate from the weak anticholinesterase action of this drug in vitro, and since the accompanying muscarine-like effects are minimal, the action of edrophonium has been attributed to a direct, selective action on neuromuscular transmission (3–5).

Patients with myasthenia gravis are more sensitive to the depressant effect of d-tubocurarine on muscle strength than normal subjects (6). Following intravenous administration the average difference in response is five fold (7). In contrast, myasthenic patients are as responsive (7) or less responsive (8–10) than normal subjects to the depressant effect of intravenous decamethonium on muscle strength. The weakness may be preceded by transient improvement in strength in myasthenic patients (7, 10). In these patients the weakness produced by decamethonium is reversed following the intravenous administration of neostigmine or edrophonium, whereas in normal subjects the weakness is intensified by these drugs (7, 10). Churchill-Davidson and Richardson have suggested that decamethonium produces transient depolarization, followed by a competitive type of block in the clinically weak muscles of myasthenic patients, and by a depolarizing type of block in normal subjects (10). This difference in the action of decamethonium led these authors, and Zaimis, to propose that a similar difference in the action of acetylcholine might occur in myasthenic patients, and that this might be the cause of the functional defect in this disease (11).

In the studies to be reported the effect in myasthenic patients of intra-

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arterially administered neostigmine, edrophonium, d-tubocurarine, and decamethonium on neuromuscular transmission and on the action of injected ACh has been investigated. The methods employed were previously described (12). Neostigmine and edrophonium produced partial or complete repair of the myasthenic defect, followed, after larger doses, by depression of neuromuscular function. The effects of these drugs appeared to be due mainly to their anticholinesterase action, although edrophonium may have had an additional direct effect. d-Tubocurarine produced more depression than in normal subjects, but decurarization by neostigmine, tetanic nerve stimulation, or ACh was no less striking. Decamethonium produced transient repair, followed by depression, which had most of the properties of a competitive block, in contrast to the non-competitive or mixed types of block which occurred in normal subjects (13).

RESULTS

Patients with Generalized Myasthenia Gravis

Anticholinesterase Compounds

Effect on neuromuscular function: *Neostigmine:* The intra-arterial administration of 0.02 to 2 mg. resulted in each of 20 patients in an increase in the amplitude of muscle action potentials evoked by nerve stimulation, with improvement or disappearance of the progressive decline (decrement) in response to successive stimuli present before drug administration (1) (Fig. 1). There was also disappearance of post-tetanic facilitation. With larger doses of neostigmine, this improvement in function was rapidly followed by progressive depression of the successive potentials of a train, sometimes accompanied by repetitive firing after the first potential, resembling the effect in normal subjects. In patients with the mildest degrees of myasthenic block, small doses of neostigmine, in some instances as little as 0.02 to 0.05 mg., produced repair of the defect, and as little as 0.2 to 0.5 mg. resulted in progressive depression and repetitive firing. In patients with severe block, larger doses of neostigmine were required to produce repair, which was frequently incomplete. In those with the most severe block, the repeated administration of neostigmine produced progressive depression of evoked potentials before they attained equal amplitude, and while they were still far below the normal range (Fig. 2). The depressant effect of large doses of neostigmine in myasthenic patients resembled the more marked depressant effect of smaller doses in normal subjects (13), except that there was less repetitive discharge after the initial potential. The reparative and depressant effects of neostigmine began approximately 15 seconds after injection, reached a maximum in one minute (Table I), and lasted for one half to several hours, depending on the amount injected. The time course of the depressant effect was approximately the same as in normal subjects.

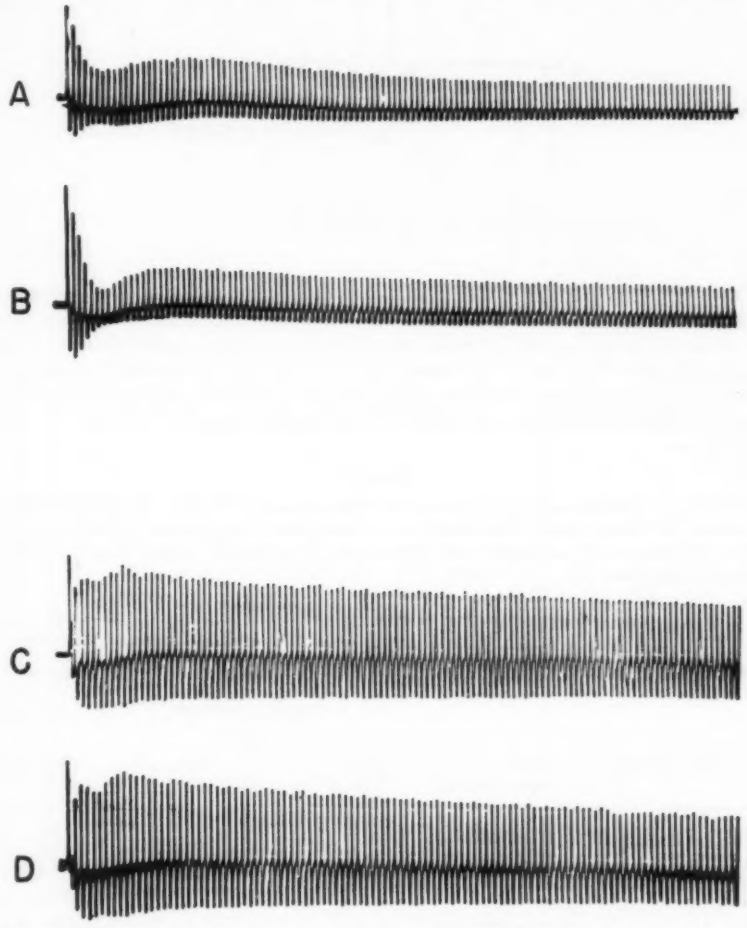

FIG. 1. Effect of neostigmine on the muscle action potential response to prolonged repetitive nerve stimulation. (Myasthenic patient, R. P.). A and B. Before neostigmine. A, response to 121 stimuli at 50 per second, showing rapid decline in amplitude of successive potentials, followed by partial recovery and then more gradual decline, characteristic of myasthenia gravis. B, response to 121 stimuli at 50 per second applied 5 seconds later, showing increase in amplitude of first four potentials (post-tetanic facilitation) and more marked decline in amplitude of subsequent potentials. C and D. After neostigmine (2 mg. intra-arterially), showing less marked decline in amplitude of successive potentials, and disappearance of post-tetanic potentiation.

Edrophonium: The administration of 0.2 to 1 mg. resulted in each of 7 patients in an increase in the amplitude of evoked potentials, with improvement or disappearance of the "myasthenic" decrement (Fig. 3), resembling the effect of comparable doses of neostigmine. Likewise, larger doses (2 to

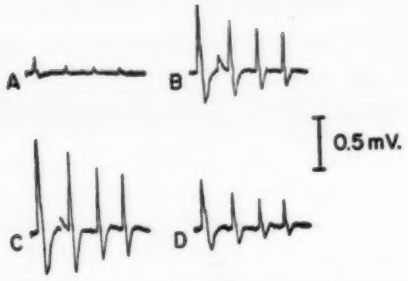

FIG. 2. Incomplete repair of the block in neuromuscular transmission by neostigmine in a patient with very severe myasthenia gravis (H. W.). A, control muscle action potentials in response to four nerve stimuli 40 msec. apart. B & C, incomplete repair following the intra-arterial injection of 2.5 and 3 mg. neostigmine. D, depression immediately following the reparative effect of the second injection. The depressant effect of large doses of neostigmine appeared before the potentials were restored to the normal range.

TABLE I

Time to Onset and Maximum Degree of Depression of Muscle Action Potentials Evoked by Nerve Stimuli Following the Intra-Arterial Administration of Neostigmine, Edrophonium, d-Tubocurarine and Decamethonium in Normal Subjects and Patients with Generalized Myasthenia Gravis, and Time to Repair in the Latter

The mean values, and standard error of the mean are recorded.

Compound and Dose (mg., mean)	Subjects	No.	Time (seconds) between Injection and			
			Repair		Depression	
			Onset ± S.E.	Maximum ± S.E.	Onset ± S.E.	Maximum ± S.E.
Neostigmine (0.9 mg.)	Normal	14			12 ± 2.4	44 ± 7.0
	Generalized m.g.	20	13 ± 1.5	65 ± 6.3	15 ± 2.6	64 ± 8.4
Edrophonium (6.5 mg.)	Normal	14			9 ± 1.1	24 ± 3.7
	Generalized m.g.	7	7 ± 1.1	43 ± 19.6	9 ± 1.3	39 ± 9.2
d-Tubocurarine (0.15 mg.)	Normal	13			13 ± 1.8	45 ± 4.9
	Generalized m.g.	6			18 ± 3.3	59 ± 9.8
Decamethonium (0.07 mg.)	Normal	10			12 ± 1.6	32 ± 4.4
	Generalized m.g.	6	10 ± 1.7	27 ± 3.5	18 ± 4.6	71 ± 12.8

12 mg.) resulted, in most patients, in depression, particularly of the latter potentials of a train. The depression was more marked than that which followed large doses of neostigmine. The action of edrophonium, whether reparative or depressant, was nearly twice as rapid as that of neostigmine in onset and time to occurrence of maximal effect (Table I), and less than one third as prolonged. The time course of the depressant effect was the same as in normal subjects. The maximum local or systemic improvement never exceeded that attained following neostigmine.

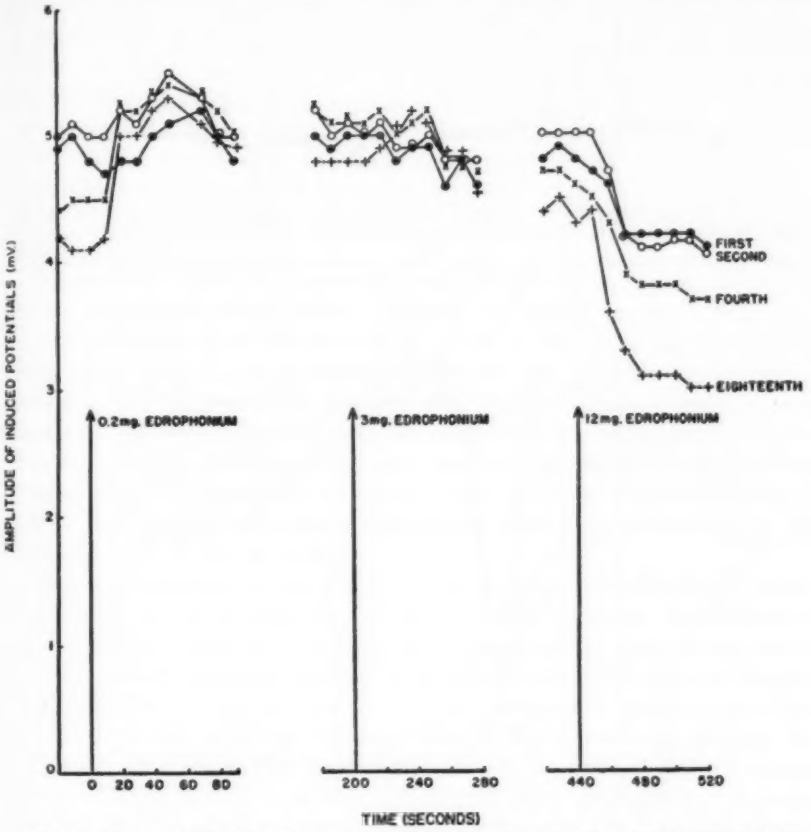

FIG. 3. Repair of the block in neuromuscular transmission by a small dose of intra-arterial edrophonium, and depressant effect of larger doses. (Myasthenic patient, R. A.). The amplitude of the muscle action potential response to the first, second, fourth, and eighteenth of a train of 18 nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted.

Effect on the Action of ACh: The prior administration of neostigmine or edrophonium resulted in an increase and prolongation of the "prompt" depressant effect of ACh on evoked potentials in each of six patients. The degree, pattern, and time course of the increase was the same as in normal subjects (13). An increase in the reparative action of small doses of ACh was also observed in three patients after neostigmine administration.

d-Tubocurarine

Effect on neuromuscular function: The depressant effect of d-tubocurarine on the initial potential of a train evoked by repetitive nerve stimuli was 5 times as great in myasthenic patients as in normal subjects, and on subsequent

FIG. 4. Depressant effect of a small dose of d-tubocurarine (0.1 mg. intra-arterially). (Myasthenic patient, R. P.). A, control muscle action potentials evoked by four stimuli 40 msec. apart. B, depression of the second, third, and fourth potentials following injection.

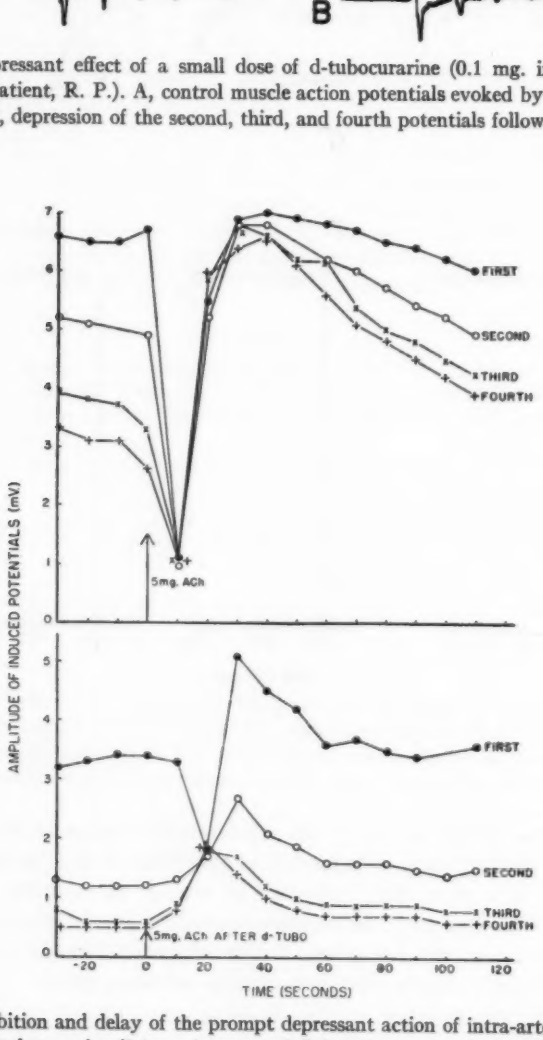

FIG. 5. Inhibition and delay of the prompt depressant action of intra-arterial ACh by a small dose of d-tubocurarine (0.1 mg. intra-arterially), and transient decurarization produced by ACh. (Myasthenic patient, P. B.). The amplitude of the muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted.

potentials ten times as great. The depressant effect varied with the degree to which the muscle tested was affected by the disease. Since the depression of the second and subsequent potentials of a train was much greater than that of the initial potential (Fig. 4), more decrement occurred than in normal subjects. The time to onset and maximum depression was the same as in normal subjects, but the rate of recovery was slower. There was marked increase in the degree of post-tetanic facilitation, indicating that the tetanus produced decurarization.

Effect on the "prompt" depressant action of ACh: The prior administration of d-tubocurarine inhibited the "prompt" depressant action of ACh to a greater degree than in normal subjects. Doses as low as 0.1 mg., which had little or no effect in normal subjects, delayed and reduced the depressant action of ACh in myasthenic patients (Fig. 5). This effect was more evident on the second and subsequent potentials of a train than on the initial potential, whereas in normal subjects the reverse was true (13). In myasthenic patients the prompt depressant action of ACh is greatest on the initial potential (Fig. 5) (14), which may account for the less marked effect of d-tubocurarine on the action of ACh on this potential.

Decurarizing action of ACh: Prior to d-tubocurarine ACh produced a greater increase in the latter potentials of a train, while following d-tubocurarine ACh produced a greater increase in the initial potential (Fig. 5). In normal subjects, the decurarizing action of ACh followed the latter pattern, so that it appears likely that the increase in the amplitude of induced potentials produced by ACh following d-tubocurarine block in myasthenic patients was due more to the decurarizing action of ACh than to repair of the myasthenic defect. The decurarizing action of ACh was more striking in myasthenic patients than in normal subjects at comparable degrees of d-tubocurarine block, probably because the former required smaller doses of this drug to elicit the same degree of block.

Decurarizing action of neostigmine: 0.1 to 2 mg. of neostigmine produced a striking increase in the amplitude of evoked potentials that had been depressed by d-tubocurarine. This effect began approximately 15 seconds after injection, became maximal in 2 to 5 minutes (Fig. 6), and, as in normal subjects, was much more marked than the action of ACh. Reversal of the d-tubocurarine block was followed by an increase in the potentials above their initial amplitude; i.e., repair of the myasthenic defect.

Decamethonium

Effect on neuromuscular function: Within a few seconds after the injection of 0.05 mg. of decamethonium there occurred a burst of motor unit activity similar to that observed in normal subjects, consisting of potentials of 50 to

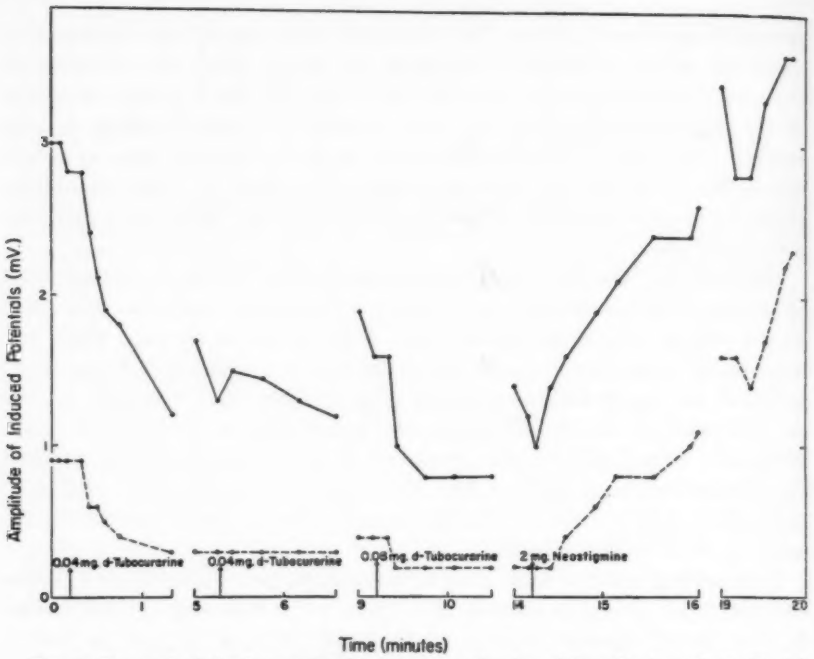

FIG. 6. Reversal of d-tubocurarine block by neostigmine. (Myasthenic patient, R. A.). The amplitude of the muscle action potentials in response to the first (●——●) and fourth (○- - -○) of a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted before and after the intra-arterial injection of each drug.

100 microvolts amplitude which could be recorded from intramuscular electrodes. The motor activity lasted for approximately five seconds. In four of six patients, doses of decamethonium from 0.025 through 0.2 mg. produced a slight increase in the amplitude of the initial evoked potential of a train, and a moderate increase in the amplitude of the second and subsequent potentials (Figs. 7 and 8). Since the increase was greatest in those potentials of the train which had the greatest degree of myasthenic block, there was considerable repair of the myasthenic decrement, although the potentials were not restored to equal amplitude. This reparative action of decamethonium began an average of 10 seconds after injection, reached a maximum in 27 seconds, and then diminished during the next 50 seconds. It was immediately followed by a more prolonged period of depression of evoked potentials (Figs. 7 and 8). The depressant effect was most marked on the fourth and subsequent potentials of a train, and was least on the initial potential, which was remarkably resistant to the effect of decamethonium. The depressant effect on the fourth and subse-

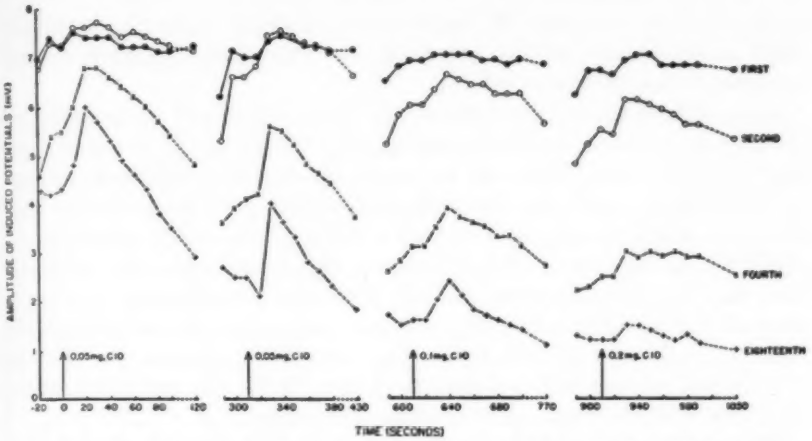

FIG. 7. Effect of successive intra-arterial injections of decamethonium (C_{10}) on neuromuscular transmission. (Myasthenic patient, R. A.). The amplitude of the muscle action potentials in response to a train of 18 nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted.

Each injection of decamethonium produced transient repair followed by depression.

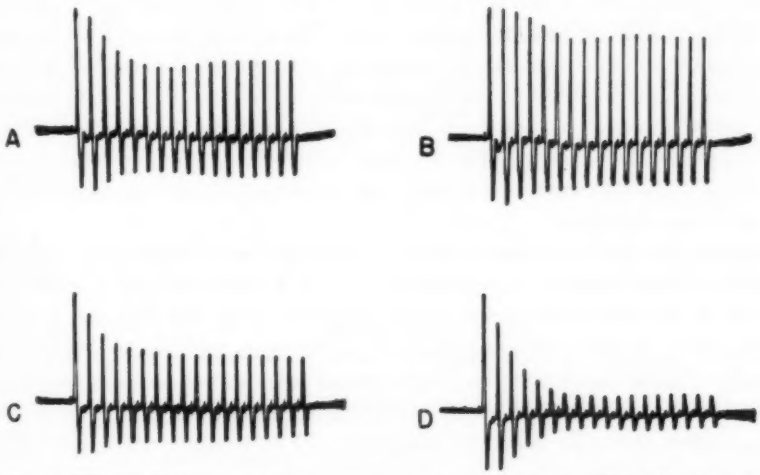

FIG. 8. Effect of decamethonium on neuromuscular transmission. (Myasthenic patient, R. A.): A, control muscle action potentials evoked by a train of nerve stimuli 40 msec. apart. B, partial repair of the myasthenic block 20 seconds after the intra-arterial injection of 0.05 mg. decamethonium. C, depression, particularly of the latter potentials of the train, 2 minutes after injection. D, further depression 1½ minutes after the injection of additional doses of decamethonium (0.4 mg. total). The time course of these effects is shown in Fig. 7.

quent potentials was the same as in normal subjects, while the depressant effect on the initial potential was much less. This resulted in more marked decrement than in normal subjects.

When a reparative effect followed the administration of decamethonium the depressant action did not become apparent for 80 seconds (average time) after injection. When repair did not occur, the depression began an average of 15 seconds after injection. The maximum depression was reached one minute later, and then gradually wore off over a period of one half to several hours, depending on the dose of drug. Depression began and reached its maximum later than in normal subjects (Table I). When the administration of decamethonium was repeated during this depression, there again was transient partial repair of the latter potentials, followed by prolonged depression (Fig. 7). The initial potential remained resistant to change. With repeated injections the reparative and depressant effects became less marked.

Effect on the action of ACh: The prior administration of decamethonium had no effect on the prompt depressant action of ACh, as measured by per cent reduction of muscle action potentials, although the decrease in amplitude (millivolts) of the already lowered potentials was less (Fig. 9). This was similar to the effect of decamethonium in normal subjects. As in normal subjects, the onset of the prompt depressant effect of ACh was delayed by about 10 seconds.

Effect of ACh on decamethonium block: The prior administration of decamethonium resulted in a slight increase in the reparative action of ACh, as measured by change in amplitude (millivolts) of the potentials, particularly of the initial potential of a train (Fig. 9). There was moderate increase as measured by per cent change. This indicates that ACh probably produced some reversal of the decamethonium block. The time course of the reparative action of ACh was unchanged.

Reversal of decamethonium block by neostigmine: Following the administration of decamethonium, neostigmine (0.5 to 2 mg.) produced a greater increase in the amplitude of the evoked potentials (Fig. 10) than when it was given alone. Further administration of neostigmine, in doses of 2 to 3 mg., usually resulted in depression of the second and subsequent potentials. In normal subjects reversal of decamethonium block occurred only after the administration of smaller doses of neostigmine (0.1 to 0.3 mg.), while larger doses intensified the block (13).

Reversal of decamethonium block by edrophonium: Edrophonium, like neostigmine, promptly reversed decamethonium block in myasthenic patients, in contrast to the increase in block which occurred in normal subjects. The administration of 0.2 to 3 mg. of this drug, following depression by decamethonium, resulted in prompt increase in the amplitude of the potentials,

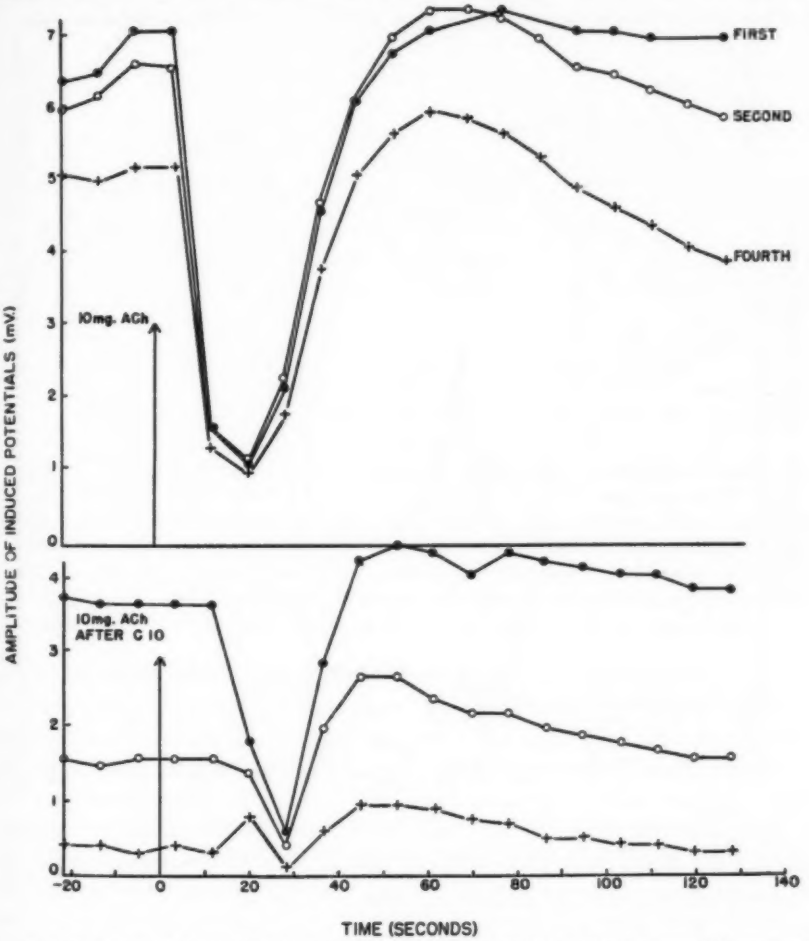

FIG. 9. Effect of decamethonium (0.15 mg. intra-arterially) on the "prompt" depressant and reparative actions of ACh. (Myasthenic patient, R. P.). The amplitude of the muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted.

The prior administration of decamethonium (C_{10}, lower block) did not affect the "prompt" depressant action of ACh, except for slight delay in onset. The reparative action of ACh was slightly increased.

particularly the latter ones of a train (Fig. 11). Both this increase, and the depression which followed larger doses of edrophonium (12 mg.), were more marked than after edrophonium alone (Fig. 3).

Inhibition of the depressant effect of decamethonium by ACh and choline:

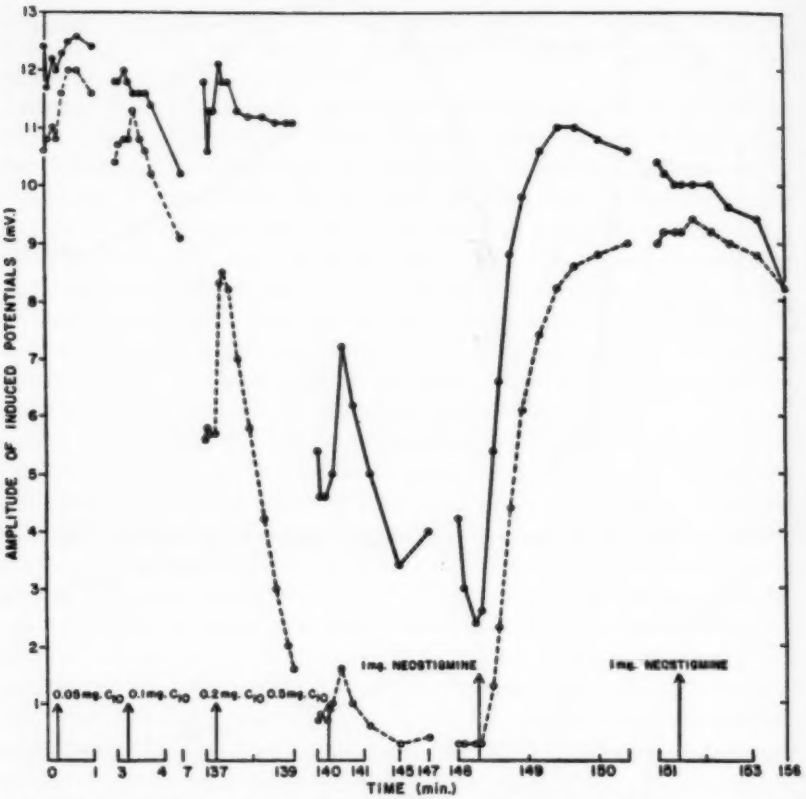

FIG. 10. Reversal of decamethonium (C_{10}) block by neostigmine. (Myasthenic patient, A. G.). The amplitude of the muscle action potentials in response to the first (●——●) and fourth (○- - -○) of a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted before and after the intra-arterial injection of each drug.

During the "late" ACh depression or the depression produced by choline, the blocking effect of decamethonium was reduced (Fig. 12). The reparative effect of the initial injection of decamethonium was increased.

Patients with Oculobulbar Myasthenia Gravis

Neostigmine: The degree of depression produced by neostimine varied considerably in these patients. In twelve patients (group I) one mg. produced the same average reduction as in normal subjects (16 per cent in the response to the first stimulus of a train and 70 per cent in the response to the fourth). There were, however, fewer fasciculations in the injected extremity and less

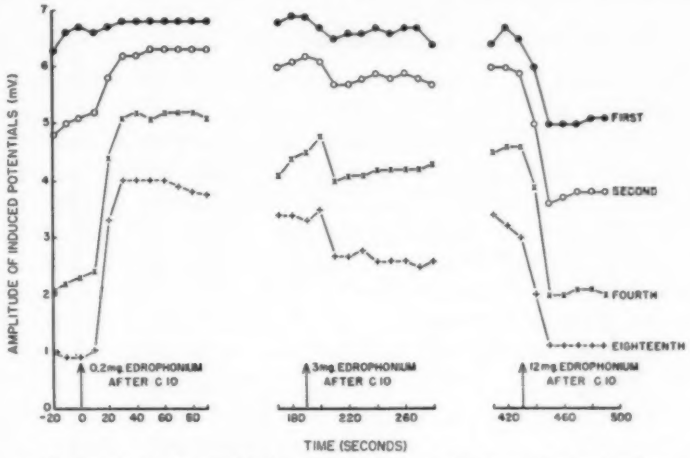

FIG. 11. Reversal of decamethonium block by a small dose of edrophonium, and depression following larger doses. (Myasthenic patient, R. A.). The amplitude of the muscle action potentials in response to the first, second, fourth, and eighteenth of a train of 18 nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted before and after the intra-arterial injection of each dose of edrophonium, following the intra-arterial injection of a total of 0.4 mg. decamethonium (C_{10}). The reparative and depressant doses of edrophonium were the same as prior to decamethonium (Fig. 3), but the degree of change was greater.

repetitive firing after the initial potential of a train. In four patients (group II) neostigmine produced less depression than in any of the normal subjects studied; i.e., a reduction of 3 to 10 per cent (average 3 per cent) in the first potential and of 5 to 20 per cent (average 16 per cent) in the fourth. In these patients neostigmine produced no fasciculations in the injected extremity, and little or no evidence of repetitive firing after the initial potential. In group I the depressant action of choline, and the effect of neostigmine on this depression, were the same as in normal subjects, while in group II the properties of the depression produced by choline were intermediate between those observed in normal subjects and in patients with generalized myasthenia gravis (14).

Edrophonium produced depression of evoked potentials in two patients similar to that observed in normal subjects.

d-Tubocurarine: In each of four patients this drug produced slightly more depression of evoked potentials than was observed in any of the normal subjects.

Decamethonium: In three of four patients this drug produced a slightly greater degree of progressive depression of the potentials of a train than normal. In one patient the administration of edrophonium following decamethonium reversed the decamethonium induced depression.

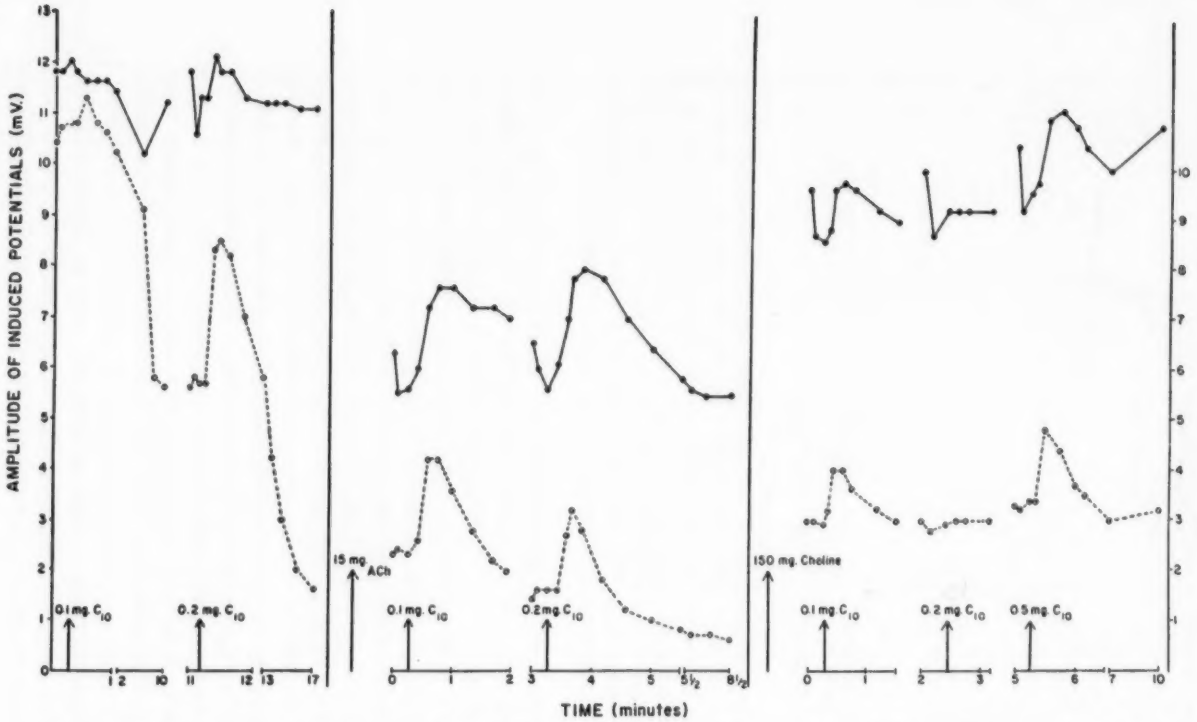

FIG. 12. Effect of the prior administration of ACh and choline on the neuromuscular action of decamethonium (C_{10}). (Myasthenic patient, J. G.).

Left: Effect of decamethonium alone.

Middle and Right: Effect of decamethonium following "late" depression by ACh (middle), and depression by choline (right). The depressant effect of decamethonium was inhibited, particularly after choline, while the reparative effect of the initial injection was increased. The amplitude of the muscle action potentials in response to the first (●——●) and fourth (○- - -○) of a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted before and after the intra-arterial injection of each dose of drug.

DISCUSSION

It is probable that the actions of neostigmine in the normal subject are attributable to its anticholinesterase effect rather than to a direct cholinergic action, because of the gradual onset of its blocking effect, the slight depression of the initial potential of a train, the progressive depression of the potentials of a train, and the potentiation of the "prompt" depressant effect of ACh (13). In patients with myasthenia gravis small doses of neostigmine produced repair of the defect in neuromuscular function, and larger doses than normal were required to produce depression of neuromuscular transmission. However, the time course of the depressant effect of neostigmine, the pattern of the depression, and the effect of neostigmine on the "prompt" depressant action of ACh were similar to that in normal subjects. These effects are more compatible with neostigmine acting primarily as an anticholinesterase agent than as a direct acting cholinergic agent.

The dose of neostigmine that produced depression of neuromuscular transmission was, in general, less in patients with mild than with severe myasthenia. In some patients with severe myasthenia, depression occurred following incomplete repair of the myasthenic defect. In these patients, the administration of increasing doses of neostigmine, or of other anticholinesterase compound, generally resulted in only limited clinical improvement; i.e., the strength of most of the affected muscles remained below normal. Further administration resulted in weakness of some muscles, particularly following the longer acting anticholinesterase agents, such as pyridostigmine (Mestinon) and ambenomium (Mytelase). These observations suggest that the neuromuscular block due to myasthenia gravis may be reversible (i.e., competitive) at some motor end-plates, and irreversible (i.e., non-competitive) or partly reversible at others. The relative proportion in any muscle of these types of block may determine the degree of improvement attained following the administration of anticholinesterase compounds, and the severity of the weakness produced by excessive doses.

The effect of *edrophonium* on neuromuscular transmission resembled that of neostigmine, except that, as in normal subjects, edrophonium had more rapid onset (Table I) and shorter duration of action. The maximum repair of neuromuscular transmission attained following this drug, and the maximum improvement in general strength, was the same as after neostigmine. Improvement of muscles which failed to respond to neostigmine (4) was not observed. Large doses of edrophonium had more depressant effect, particularly on the initial potential of a train, than did large doses of neostigmine. The effect of edrophonium on the prompt depressant action of ACh was similar to that of neostigmine. These effects of edrophonium are compatible with its acting primarily as an anticholinesterase agent, although its more rapid action and

increased. The amplitude of the muscle action potentials in response to the first (●——●) and fourth (○- - -○) of a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted before and after the intra-arterial injection of each dose

greater depressant effect on the initial potential of a train suggest that there may be an additional direct effect on neuromuscular transmission.

d-Tubocurarine not only produced greater depression of evoked potentials in myasthenic patients than in normal subjects, but it also caused more marked inhibition of the "prompt" depressant (depolarizing) action of ACh. In the myasthenic this inhibition was more marked in the latter potentials of a train than in the initial potential, whereas in the normal the reverse was true (13). Thus, d-tubocurarine not only blocks the action of ACh on the end-plates to a greater degree in myasthenic patients than in normal subjects, but this effect is increased following neuromuscular transmission to a greater degree. These results are compatible with a competitive (ACh inhibitory) block in the myasthenic which is enhanced by the competitive blocking action of d-tubocurarine.

The decurarizing action of ACh in myasthenic patients was greater than in normal subjects, probably because smaller doses of d-tubocurarine were required to produce a comparable degree of block. This effect of ACh appeared to be due more to decurarization than to repair of the myasthenic defect, since ACh produced more repair of the initial potential of a train. Decurarization by neostigmine and by tetanic nerve stimulation was as great as in normal subjects. These findings suggest that the site of the d-tubocurarine block may be proximal to that of the myasthenic block.

The observations that have been described on the effects of intra-arterially administered *decamethonium* in myasthenic patients were, in general, similar to those described by Churchill-Davidson and Richardson (10) following intravenous administration. Decamethonium produced a burst of motor unit activity, followed by transient partial repair of the myasthenic defect, and then by prolonged depression. The depression had the properties of a competitive block, in contrast to the predominantly non-competitive block which the same doses of this drug produced in normal subjects (13). The degree of progressive depression of the responses to repetitive nerve stimulation was much more marked than in normal subjects, and the decamethonium block was promptly reversed by neostigmine or edrophonium, or, to a lesser extent, by ACh. However, in contrast to the competitive type of block produced by d-tubocurarine, there was no inhibition of the "prompt" depressant (depolarizing (15)) action of ACh. The complex nature of the decamethonium block is also illustrated by the finding that this compound potentiated not only the reparative action of edrophonium in myasthenic patients, but also the depressant action of large doses of this drug. This effect does not appear to be due to the slight anticholinesterase activity of decamethonium (1/100 that of neostigmine) (16), since there was no potentiation of the "prompt" depressant action of ACh.

The more marked decrement produced by decamethonium in myasthenic

patients was due to resistance to depression of the initial response to a train of repetitive nerve stimuli. The subsequent responses were depressed to the same degree as in normal subjects. The initial response was also more resistant than subsequent responses to the depressant effect of d-tubocurarine, choline, the "late" effect of ACh, and worsening of the myasthenia. It is also less responsive to the reparative action of decamethonium, ACh, and choline (14). The transmission of the initial stimulus across the neuromuscular junction alters the properties of the response to subsequent stimuli. Most of the changes that occur after decamethonium are compatible with an increase in the competitive (ACh inhibitory) block that is characteristic of myasthenia. The resistance of the initial potential of a train to decamethonium may be due to the mixed nature of the block produced by this compound in myasthenic patients, and to the minimal degree of myasthenic block present before nerve stimulation in all but the most severely involved muscles (14). It is of interest that a similar type of resistance is seen in some species in which decamethonium has a dual mode of action, initially depolarizing and then competitive, with the actions being antagonistic (17). The finding of Churchill-Davidson and Richardson (10) that the intravenous administration of decamethonium to myasthenic patients reduced grip strength without reducing the action potential response to nerve stimulation is doubtless due to the low frequency of stimulation that was employed (one per second). At this frequency, each action potential is comparable to the initial potential of a train, and is, therefore, resistant to the depressant effect of decamethonium. The grip strength is more analogous to the response to repetitive nerve stimulation at a higher frequency. In this situation the potentials are progressively depressed by decamethonium.

In the patients with oculobulbar myasthenia who were investigated, there was no evidence of abnormal tolerance of the clinically uninvolved muscles studied to the depressant effect of decamethonium, in contrast to the findings of Churchill-Davidson and Richardson (10). Increased tolerance was observed to the depressant effect of choline (14). The observation that edrophonium reversed decamethonium block in a clinically uninvolved muscle of a patient with oculobulbar myasthenia indicates a latent defect in neuromuscular transmission. The effects of neostigmine, d-tubocurarine, and choline (14) have provided additional evidence for the presence of such a defect in many of these patients.

The effects of intra-arterial decamethonium in normal subjects (13) and myasthenic patients were in general similar to those of choline (14). Both compounds had two stages of action in myasthenic patients, producing transient repair followed by depression of neuromuscular transmission, attributable to a transient depolarization followed by a more prolonged and predominantly competitive block. Both compounds had less depressant effect on the initial

potential of a train than on subsequent potentials, but only decamethonium had less effect on the initial potential than in normal subjects. The compounds also differed in that decamethonium had the same depressant effect on the latter potentials of a train in myasthenic patients and normal subjects, while choline had a greater depressant effect in the myasthenic. This difference may be related to the failure of decamethonium to inhibit the "prompt" depressant (depolarizing) action of ACh in either group. In contrast, choline inhibited this action of ACh in myasthenic patients, although not in normal subjects. These findings suggest that the site of the blocking action of decamethonium in the myasthenic may be distal to the site of the "prompt" depressant action of ACh, while that of choline may be proximal to this site.

The abnormal response of myasthenic patients to decamethonium, and choline, could be due to an abnormal response of their end-plates to these compounds, or to their conversion at the myoneural junction into competitive blocking agents. Decamethonium may be converted from a depolarizing blocking agent into a competitive blocking agent by replacement of one of the methyl radicals on each nitrogen by aromatic groups, such as nitrobenzyl radicals, or by conversion to tridecamethonium (18). Likewise, in a series of bis-quaternary bipiperidine derivatives the compounds having methyl groups on the nitrogens are depolarizing blocking agents, while the compounds having aromatic groups on the nitrogens are competitive blocking agents (19). Churchill-Davidson and Richardson observed that the rate of urinary excretion of decamethonium was approximately the same in myasthenic patients as in normal subjects, which they interpreted to indicate that the decamethonium was probably not converted into another compound in the former. However, since they recovered only 40 per cent of the amount injected in both groups, this possibility cannot be excluded.

SUMMARY

1. In patients with myasthenia gravis the intra-arterial administration of neostigmine or edrophonium produced an increase in the amplitude of muscle action potentials evoked by nerve stimulation, followed, after large doses, by depression. There was an increase in the reparative and depressant effects of ACh. This is compatible with action mainly as anticholinesterase agents. Edrophonium had more rapid onset of action than neostigmine, suggesting that it may have an additional direct effect on neuromuscular transmission.

2. d-Tubocurarine produced greater depression of evoked potentials in myasthenic patients than in normal subjects, and more marked inhibition of the "prompt" depressant (depolarizing) action of ACh. However, decurarization by neostigmine, tetanic nerve stimulation, and ACh was no less striking than in normal subjects. This suggests that the site of the d-tubocurarine block may be proximal to that of the myasthenic block.

3. Decamethonium produced a transient increase in the amplitude of evoked potentials in myasthenic patients, attributable to depolarization, followed by progressive depression of successive potentials. The initial potential of a train was depressed to a lesser degree than in normal subjects, while subsequent potentials were depressed to the same extent. The depression had most of the properties of a competitive block, including reversal by ACh, neostigmine, or edrophonium. The depression developed more slowly than in normal subjects.

ACKNOWLEDGMENTS

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METHYLCELLULOSE AS A WETTING AGENT IN BLOOD CLOT[1, 2]

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The rapidity of blood coagulation and the physical properties of the resulting clot are governed largely by the concentrations of the various substrates and factors that enter into the clotting reaction. Plasma constituents other than those participating directly in the reaction chain may also influence clot characteristics. For example, a clot formed in plasma exhibits greater strength and elasticity and is less soluble in urea than one prepared from pure fibrinogen (1). Ellicott and Conley (2) showed that the serum constituent which acts in conjunction with platelets to promote retraction of fibrinogen clot is not a specific factor and can be replaced by various proteins and by a foreign colloid, gum acacia.

The present study is concerned with the effect of several colloids, added *in vitro*, on certain physical properties of blood plasma, both in the clotted and fluid state. Of the various materials tested, methylcellulose stood out uniquely as a surface active agent. It alters some of the clot characteristics in a direction that might be favorable for hemostasis. The clot surface takes on hydrophilic properties in contrast to its normal hydrophobic character. The increased wettability of the surface facilitates spread and adherence of superimposed layers of clot. In fluid plasma this surface activity can be detected and measured by a pronounced effect on surface tension.

METHODS AND MATERIALS

Wetting Action. Human venous blood was drawn in a siliconed syringe containing heparin in the amount of 2 microgm. per ml. of blood. Plasma was separated and stored in silicone coated glassware, and was kept refrigerated at 1°–4°C. Coagulation was induced by thromboplastin (Difco), prepared by extracting 150 mg. of the lyophilized rabbit-brain material at 47°C. with 5.0 ml. of saline and filtering the suspension through a loose plug of absorbent cotton.

A mixture of plasma, 0.45 ml., and colloid solution, 0.05 ml., was flowed over the interior lower-half of a culture tube and the excess was drained to the bottom. 0.05 ml. of thromboplastin solution was added, the mixture was again flowed over the previously moistened sur-

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[2] Preliminary reports were presented at meetings of the Federation of American Societies for Experimental Biology. Federation Proceedings, 1955, **14:** 383 and 1956, **15:** 476.

face, and the excess was withdrawn by pipette. The tube was held vertically a minute or two to permit formation of a uniform film during coagulation. Then with the tube placed horizontally, a drop of fluid plasma was gently deposited on the surface of the clot with a bent-tip pipette and the extent of spread was noted.

Surface Tension Measurement. Human plasma was separated from citrated blood-bank samples. Measurements were made with a Cenco-du Noüy tensiometer. The 4 cm. platinum ring was rinsed with distilled water and was brought to incandescence in a bunsen flame before each test. The instrument was calibrated with standard weights and was checked by measurements on doubly distilled water. All glassware was soaked in warm detergent, thoroughly rinsed in distilled water, and oven dried at 150°C.

Various types of methylcellulose were generously supplied as Methocel by the Dow Chemical Company, Midland, Michigan. National Formulary grade, viscosity types 10 and 400 centipoises (cps.), were used in this study. The Hercules Powder Company, Wilmington, Delaware, very kindly supplied carboxymethylcellulose as Cellulose Gum (CMC) in a variety of types. Premium grade, CMC-70-low viscosity (25–50 cps.), and premium grade, CMC-70-medium viscosity (300-600 cps.) were tested. Other colloid plasma volume expanders were obtained commercially: PVP-Macrose, Schenley Laboratories, containing 3.5 per cent polyvinylpyrrolidone; Knox Special Gelatine Solution, Intravenous 6 per cent, Knox Gelatine Protein Products, Inc.; Expandex, Dextran Injection, 6 per cent, C.S.C. Pharmaceuticals.

RESULTS

In the absence of an additive, a drop of fluid plasma deposited on the clotted surface remains associated as a lens like droplet, Figure 1. When methylcellulose is present in the clot, the drop spreads quickly over the surface. Such spread may be expressed in terms of contact angle. Complete wetting obtained in the presence of methylcellulose corresponds to a contact angle of 0°. Without the

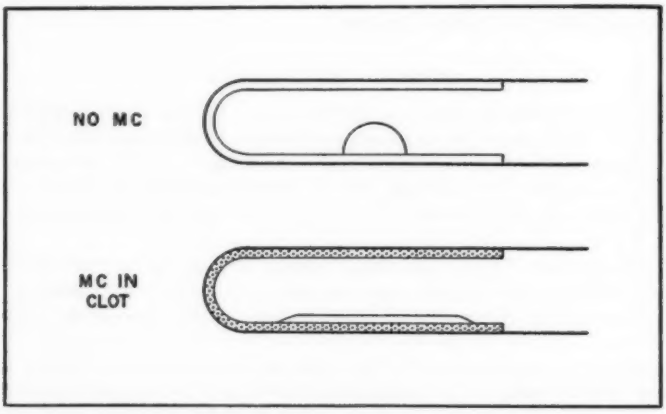

FIG. 1. Diagrammatic sketch of clot-wetting test. − denotes absence of wetting action. Plasma remains associated as lens like droplet with a contact angle of approximately 90°. + denotes positive wetting action. Plasma spreads over clot surface with contact angle of 0°.

wetting agent the liquid lens gave a contact angle of approximately 90°. As the surface of the clot became slowly moistened this value gradually diminished.

As shown in Table I, none of the colloids tested promoted spreading in a degree comparable to methylcellulose. The colloid volume expanders, gelatin, polyvinylpyrrolidone, and dextran showed no wetting action in 50 to 100 times the concentration found to be effective with methylcellulose. Carboxymethylcellulose was similarly inactive. The more viscous type of methylcellulose, designated as 400 cps., was about equally or slightly less effective than the low viscosity, 10 cps., material.

TABLE I

Wetting Action of Colloid Additives in Clotted Blood Plasma

Colloid added	Concentration of colloid in clot mg./100 ml.		
	100	200	600
Methylcellulose—10 cps	+	+	
Methylcellulose—400 cps	+	+	
Carboxymethylcellulose—35 cps	−	−	
Dextran	−	−	−
Gelatin	−	−	−
Polyvinylpyrrolidone	−	−	−*
	1	5	10
Methylcellulose—10 cps	−	+	+
Methylcellulose—400 cps	−	±	+

* This concentration was 350 mg./100 ml.

TABLE II

Surface Tension Changes Induced by Colloid Additives

Medium	Colloid added	Concentration mg./100 ml.	Surface tension—dynes/cm.		
			5 min.	15 min.	30 min.
Water	Control—no colloid		73	73	73
Water	Methylcellulose—10 cps	100	56	57	57
Water	Methylcellulose—10 cps	200	55	56	56
Plasma	Control—no colloid		51	51	51
Plasma	Methylcellulose—10 cps	100	57	62	72
Plasma	Methylcellulose—10 cps	200	59	72	78
Plasma	Methylcellulose—400 cps	20	57	72	89
Plasma	Methylcellulose—400 cps	100	63	108	165
Plasma	Carboxymethylcellulose—35 cps	100	50	51	51
Plasma	Carboxymethylcellulose—35 cps	200	52	52	52
Plasma	Carboxymethylcellulose—400 cps	200	50	51	51
Plasma	Dextran	600	52	51	51
Plasma	Gelatin	600	51	52	52
Plasma	Polyvinylpyrrolidone	350	51	52	52

It should be noted that spreading action was observed only when the wetting agent was incorporated in the clotted film. When the film was left untreated and methylcellulose was added to the fluid droplet there was no increased spread. A firm clot with a smooth dry surface was essential for these tests. The principal precautions required to obtain this were the use of freshly drawn plasma, avoidance of excessive heparinization, and use of a potent thromboplastin.

Surface tension effects are shown in Table II. Control measurements on a sample of citrated human plasma gave a value of 51 dynes per cm. When methylcellulose was added, the surface tension readings rose progressively with time reaching a plateau level in about one-half hour with the 10 cps. type. More pronounced effects were obtained with 400 cps. methylcellulose. Surface tension levels were reached which were far higher than the value of 73 dynes per cm. recorded for pure glass distilled water. In contrast to its behavior in plasma, methylcellulose has a surface tension lowering effect when added to water. In a concentration of 100 mg. per 100 ml. it reduced the surface tension from 73 to 57 dynes per cm. No effect on plasma surface tension was obtained with the hydrophilic colloids, gelatin, polyvinylpyrrolidone, and dextran. Similarly, the related derivative, carboxymethylcellulose, was inactive.

DISCUSSION

Methylcellulose confers a water attracting character to the clot surface, which normally is water repellent. This is demonstrated in the simple experiment in which an aqueous droplet remains aggregated as a fluid lens on the surface of normal clot, whereas it spreads with great avidity over a methylcellulose treated coagulum. The colloid additive serves as a clot-wetting agent, but only when incorporated in the clot; it is inactive in this respect when added exclusively to the liquid layer.

Better wetting indicates increased adhesion between a liquid and solid surface. If the fluid plasma should undergo coagulation after spreading, more intimate bonding should ensue between the layers. Clot that is laid down *in vivo* as part of the hemostatic mechanism is no doubt built up by some such process of accretion. Improved wetting might thus lead to better adherence between the superimposed strata and result in improved clot structure.

Although methylcellulose is not a powerful wetting agent its chemical structure does satisfy the minimal requirements for such action (3). The basic structure is shown in Figure 2. Each anhydroglucose unit in the parent cellulose polymer contains three reactive hydroxyl groups permitting a maximum substitution of 3.0. The degree of substitution is approximately 2.0 in methylcellulose and 0.7 in the "regular" type of carboxymethylcellulose (4). The methyl groups are lipophilic in character, the remaining hydroxyl and oxygen

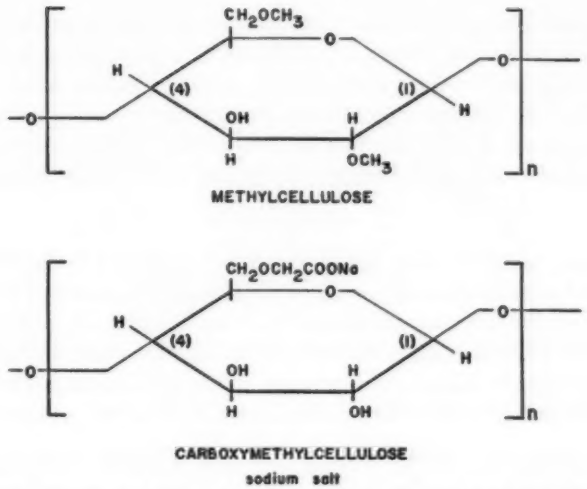

FIG. 2. Basic structures of methylcellulose and carboxymethylcellulose. Substitution can take place at any of the three hydroxyl groups in the anhydroglucose unit.

groups are polar substituents with hydrophilic properties. A molecule such as this would be adsorbed and oriented at an oil-water interface with the methyl groups anchored in the lipid and the polar groups acting as water attracting centers. In carboxymethylcellulose the lipophilic methyl groups are replaced by the polar sodium carboxymethyl. In the absence of the lipid anchoring groups, carboxymethylcellulose does not display clot-wetting activity despite many other similarities to methylcellulose. Apparently, the other hydrophilic colloids, gelatin, dextran, and polyvinylpyrrolidone also lack the structure necessary for the clot-wetting action.

It is highly significant that the wetting action in solid clot is paralleled by elevation of surface tension in fluid plasma. Here, too, methylcellulose stood out uniquely. No such surface activity was observed with any of the other colloids. There is a superficial resemblance between the action of methylcellulose and that of sodium oleate in serum. With oleate, du Noüy (5) observed a reduction of surface tension followed by a rise toward the initial value. He explained the rise by adsorption of the oleate on the proteins in the interior of the serum phase with consequent removal from the surface layer. With methylcellulose no initial fall was observed in the earliest readings, taken at 1 to 2 minutes. The surface tension rose slowly from the value in pure plasma of 51 dynes per cm. In the case of the 400 cps. material, it attained a level of 165 dynes per cm. which far exceeds the surface tension of pure water (73 dynes per cm.). It is difficult to account for this behavior in the complex colloid solution, especially

since methylcellulose has a surface tension lowering action in water. There may be interaction with some plasma constituent and accumulation of the product at the liquid surface to form a very stable surface film. Elevation of surface tension in itself would retard the spread of a liquid over a solid surface. On the other hand, should the surface film be hydrophilic it would promote spread over the aqueous, moistened surfaces such as are encountered in body tissues.

SUMMARY

Plasma clot, which normally is water repellent, acquires a hydrophilic surface when treated with methylcellulose. In fluid plasma this colloid gives rise to a slowly progressing elevation of surface tension. Neither effect was observed with the other colloids tested; namely, gelatin, dextran, polyvinylpyrrolidone, and carboxymethylcellulose. Incorporation of methylcellulose in blood plasma offers the possibility of favorably influencing clot structure and adhesiveness.

The author is indebted to Miss Dorothy Ann Wood for able technical assistance.

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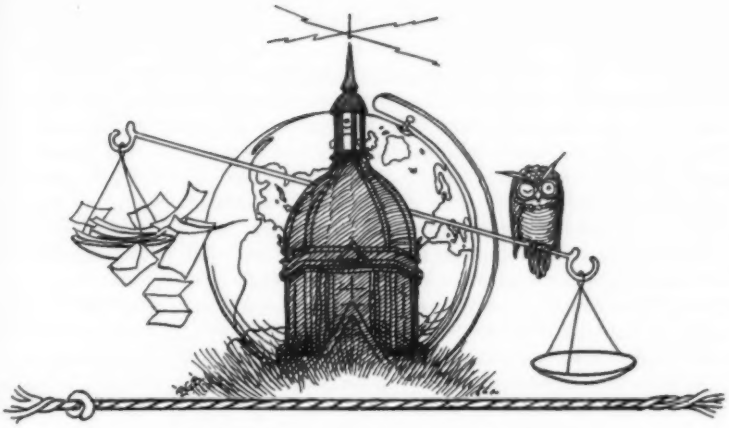

BOOK REVIEWS

Aspects of the Anatomy, Biochemistry and Pathology of the Nervous System. A symposium dedicated to Sir Charles Putnam Symonds. Guy's Hospital Reports, Vol. 105, No. 1, 1956, 150 pp.

When Sir Charles Symonds retired from the active staff at Guy's, several of his hospital colleagues delivered a series of lectures in expression of their admiration and affection for him. Selections from this series, dealing with certain chemical and pathological aspects of demyelinating and related processes, have been collected together in this special number of the Reports. Tissue culture studies of neural elements, allergic encephalomyelitis, neurotropic viruses, lipid and intermediary carbohydrate metabolism, all come under brief but clarifying review by important contributors to their respective subjects. It is particularly useful for the reader, interested in the nervous system but woefully unfamiliar with details of work in these various fields, to have them reviewed so successfully for him in such a polished manner.

It is delightful to sense the warmth of feeling of the contributors for Sir Charles, and to appreciate the choice of material bearing on a broad field of neurological medicine in which he has long been particularly interested. This is also expressed, though briefly, in a charming tribute to him which introduces the symposium—a tribute which would be warmly seconded by many in this country, and perhaps particularly in this school which lays a partial claim on him.

J. W. MAGLADERY

Adrenal Function in Infants and Children: A Symposium. Edited by LYTT I. GARDNER. 221 pp., $6.75. Grune and Stratton, New York, N. Y.

This symposium is a valuable synthesis of various significant approaches to the problems of adrenal function in infancy and childhood. Adrenal function in infancy appears to differ quantitatively and to some extent qualitatively from that of the adult. The symposium begins with a study of the histophysiology of the rat adrenal, in which Deane reports evidence of a neonatal regression of the adrenal cortex in rats, presumably due to the withdrawal of maternal ACTH at birth. Although there are probably qualitative differences between the neonatal adrenals in rats and humans, it is gratifying to find this degree of similarity

in the behaviour of the adrenal at birth in the two species. Evidence that the steroids produced by the human adrenal at birth differ from those of the mother is presented by Migeon, and indirectly substantiated by others. The response of the infant to ACTH appears to be not inferior to that of the adult, according to Klein and Rovanek, although the corticoids are known to be very low in early infancy. Judgment as to the clinical significance of this low corticoid level was withheld. Data of Kelley indicate differences between the plasma 17-hydroxycorticoids in normal children and in those with various diseases, these steroids being low in glomerulonephritis, rheumatoid arthritis and established rheumatic fever, and very high in acute illnesses and salicylate intoxication.

Wilkins opens a discussion on the present status of the therapy of congenital adrenal hyperplasia, and there are several papers on the steroid metabolism of the adreno-genital syndrome. Joan Hampson and Money present their very enlightening psychological studies pertaining to the management of sexually ambiguous cases, studies which have, however, much broader significance and merit the attention of all psychologists and psychiatrists. The normal rate of urinary excretion of sodium retaining factors, presumably aldosterone, is described by Luetscher, who finds that normal young children excrete aldosterone at a rate not much inferior to that of adults, whereas the 17-ketosteroid excretion in early childhood is very much less than in adults. Aldosterone excretion is stimulated by sodium depletion, but not by ACTH, and the aldosterone output is increased by an unknown factor common to several diseases. Sobel describes the effects of testosterone and cortisone on growth and skeletal maturation. Altogether the volume includes 15 papers, with records of discussions, bibliographies and index, and comprises well selected material of interest to pediatricians, general practitioners, endocrinologists and urologists, as well as to physiologists and students of normal development. My chief criticism is that the proceedings of a conference which took place in November, 1954, was not published until April, 1956.

EVELYN HOWARD

Textbook of the Nervous System, 2nd edition. By H. CHANDLER ELLIOTT. 437 pp., $9.00. J. B. Lippincott Co., Philadelphia, Pa.

The introduction by Dr. Wilder Penfield is inspiring and complimentary to the textbook. The preface to the second edition mainly outlines the reason for the second edition and how it differs from the first edition. The greatest difference is in the atlas which has three times the number of sections. Great emphasis is placed on the photograph and key technique of the atlas. Numerous cross references from text to atlas and from illustrations to atlas have been made. The author emphasizes that this is a student's textbook and not a reference for the specialist. Chapter 9 titled "The Neurons" has been rewritten. The dermatome charts were revised in keeping with the work of Dr. Keegan.

The textbook is written in two phases or parts. Part one surveys the field and subject simply and briefly as a whole. Part two gives a more complete and accurate account. There are 24 chapters, with a summary at the end of each chapter. The first 7 chapters take up the nervous system in outline and the last 17 as the nervous system in detail.

The author has made an effort to first understand the best method of presenting material before writing. He has made excellent use of 3-dimensional diagrams and exploded illustrations. He has freely used color for emphasis with good effect. The bibliography is excellent for the student level.

This text covers the rudiments in an understandable and readable fashion. Students using this text may wish to have other neuroanatomy texts for reference and detail.

GEORGE W. SMITH

Anesthesia for Obstetrics. Labor-Delivery-Infant Care. By ROBERT A. HINGSON AND LOUIS M. HELLMAN. (With a section on Maternal and Infant Safeguards by Virginia Apgar). 344 pp., $12.50. J. B. Lippincott Co., Philadelphia, Pa.

The relationships of the anesthesiologist and obstetrician have not been presented in a similar comprehensive form before. The material of obstetrics is of interest and importance to the anesthesiologist. The material on anesthesia is not a basic teaching text for students, young anesthesia residents or obstetricians. The organization of the book is confused by lengthy repetitions (sentences and paragraphs recur verbatim in other chapters). The pages on anesthetic techniques—with the exception of the one on caudal anesthesia—are only of passing interest to the trained anesthesiologist. The student will appreciate the section on anatomy and physiology which is well illustrated and documented.

For the clinician the highlight of the book is the section on maternal and infant safeguards by V. Apgar (which also includes a chapter on cardiac resuscitation by C. S. Beck): valuable practical information is presented in a concentrated and direct form. Interesting is the emphasis on bone marrow transfusion. Local infiltration anesthesia for cesarean section in acute hemorrhage is considered as "too time consuming to obtain a live baby"; cyclopropane anesthesia is recommended for such situations, as well as for excessive uterine spasm. The management of the parturient who has eaten recently and airway problems in general receive here the attention which they deserve.

Doctor Apgar's appeal for expert infant resuscitation is of interest: ". . . a declining infant mortality rate is related directly to the presence of trained personnel who can immediately ventilate depressed infants or intubate them if necessary . . . pressures below 12 cm. of water probably cannot expand collapsed alveoli . . . can be expanded safely by sudden short high pressure resuscitation. . . . The efficacy of these high pressure methods in the human being is unknown, but it is our feeling that these may be more effective than anything hitherto tried" (pg. 305, 306).

An abundance of material is presented concerning maternal and fetal mortality and morbidity. ". . . most investigations of the relationship of pain relief to fetal death have failed to yield conclusive answers." The present knowledge on hypoxic episodes causing irreversible damage to the infant (cerebral palsy a.o.) is discussed in detail. Infant pulmonary pathology is beautifully presented and illustrated.

The pharmacology of individual anesthetic and amnesic agents is presented in form of diagrammatic illustrations (pg. 40–56), which can mislead the student and obstetrician and are of no interest to the anesthesiologist. Why list "oxygen" and certain drugs as "antidotes for overdosage" of inhalational anesthetics? The rapid removal of the agent from the lungs by artificial pulmonary ventilation with an open system would be better teaching. Similar lack of didactic principles can be observed in other chapters. After having read the excellent comments on "oxygen in obstetrics" the reader is surprised about "80–85 per cent nitrous oxide or ethylene" (pg. 151). Also, how can the author maintain a safe oxygen concentration with ethylene when using a "closed system"? (pg. 152). With these agents denitrogenation of the lungs with 100 per cent oxygen prior to induction of anesthesia and high flowrates of gases into a semiclosed or non-rebreathing system should have been stressed. Dosages of chlorpromazine are mentioned without the route of administration (pg. 24, 160).

Continuous regional anesthetic techniques are described in great detail. Scant attention is paid to spinal anesthesia by the single injection technique (small gauge needle), the high incidence of postspinal anesthesia headache with the use of continuous spinal anesthesia (large gauge needle) and to cyclopropane in general. The space devoted to the "Western Reserve Portable Miniature Anesthesia Machine" for cyclopropane anesthesia and resuscitation is out of proportion. There is a long chapter on chloroform. Commensurate with

their importance the following points were stressed by the authors repeatedly: (1) "... the safety of the method selected depends more upon the judgement and the skill of the anesthetist than upon the drug or the technic." (2) The need for an anesthesia service in obstetrics provided by physician anesthesiologists on a 24-hour rotation basis, directed by an expert who devotes full time to this specialty.

The authors speak with conviction. Some chapters are delightful reading, for instance the one on historical perspective.

The print is clear and most illustrations are excellent. Much thought is given to diagrammatic presentations. Some tables could be simplified.

There are errors in spelling, printing and numbering of figures. The oxygen concentration in atmosphere at 8000 ft. altitude is not 15 per cent.

The bibliography seems adequate except for section four. When work of others is mentioned in the text, references are sometimes missing.

PETER SAFAR

BOOKS RECEIVED FOR REVIEW

A Doctor's Marital Guide for Patients. By BERNARD R. GREENBLAT. 88 pp., $1.50. The Budlong Press, Chicago, Ill.

A Doctor's Marital Guide for Patients, Catholic Edition. By BERNARD R. GREENBLAT. 88 pp., $1.50. The Budlong Press, Chicago, Ill.

Medical Effects of the Atomic Bomb in Japan. National Nuclear Energy Series, Division VIII, Vol. 8. Edited by ASHLEY W. OUGHTERSON AND SHIELDS WARREN. 477 pp., $8.00. McGraw-Hill Book Co. Inc., New York, N. Y.

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